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Meiosis, Mitosis, and the Induction of Chromosomal Abnormalities in Hymenolepis diminuta by Gamma Rays

Raymond Louis Kisner
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Raymond Louis Kisner entitled "Meiosis, Mitosis, and the Induction of Chromosomal Abnormalities in Hymenolepis diminuta by Gamma Rays." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in .

C. Ujors, Major Professor

We have read this dissertation and recommend its acceptance:

J.C. Howell, Helen L. Hard

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 14, 1961

To the Graduate Council:

I am submitting herewith a thesis written by Raymond Louis Kisner entitled "Meiosis, Mitosis, and the Induction of Chromosomal Abnormalities in Hymenolepis diminuta by Gamma Rays." I recommend that it be accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, with a major in Zoology.

Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:

Dean of the Graduate School

MEIOSIS, MITOSIS, AND THE INDUCTION OF CHROMOSOMAL ABNORMALITIES IN HYMENOLEPIS DIMINUTA BY GAMMA RAYS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

Raymond Louis Kisner

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INTRODUCTION

There are few reports in scientific literature concerning any type of radiation effect upon the parasitic worms, particularly cestodes. The majority of reported experiments involve the use of nematodes, especially Trichinella spiralis (Tyzzer and Honeiji, 1916; Schwartz, 1921; Levin and Evans, 1940, 1942; Honess, 1940; Evans, Levin, and Sulkin, 1941; Alicata and Burr, 1949; Alicata, 1951; Gould, Van Dyke, and Gomberg, 1953). Other species for which radiation data are available include Schistosoma mansoni (see Watts and McConnell, 1951), Ascaris spp. (in Holthusen, 1921; Dognon, 1925; Seide, 1925; Cook, 1939; Bauer and LeCalvez, 1944; Bachofer and Pahl, 1955), Ascaridia galli (see Babero, 1952), Strongyloides papilosus (see Katz, 1956), Schistosomatium douthitti (in Keefe, 1956), and Trichostrongylus axei (see Ciordia, 1957). The body of literature which specifically describes cestode irradiation is much smaller (Palais, 1933; Kisner, 1957a, 1957b; Schiller, 1957, 1959; Kuhlman, 1960; Dvorak, 1960). The present paper deals with cestode irradiation, but the above mentioned papers on nematodes provide some information which may be applicable to irradiated cestodes.

The above papers deal chiefly with morphology, including some studies of chromosome effects. The nematodes,

at least those cited, exhibited significant morphological changes only after exposure to relatively large doses of x-rays or gamma rays (400-750,000 r). Sensitivity depended upon the type and age of the tissue irradiated. Reproductive organs and embryos were most sensitive. The very large doses were required to retard growth and to prevent reproduction or the establishment of parasites within their hosts. Results were reported in terms of gross morphological change in most experiments. Some stage in the life cycle was irradiated, and a later, more mature stage was examined for abnormalities. Few investigators indicated that a cytological approach was employed. Several mentioned that irradiated embryos were delayed or stopped in cleavage stages, and that sometimes egg and sperm production was affected. The chromosomes of irradiated Ascaris spp. have been observed (Holthusen, 1921; Bauer and LeCalvez, 1944). Insofar as the author is aware, the chromosomes of an irradiated cestode have been examined only once. Dvorak (1960) observed the chromosomes of early cleavage stages in irradiated Hymenolepis nana.

Palais (1933) was probably the first to investigate the effects of radiation upon a cestode. She subjected the eggs of Hymenolepis diminuta to x-rays and ultra-violet light. The adult stage was examined for morphological abnormalities. There were no detectable changes that could

be attributed to radiation. Kisner (1957a) using the same species, exposed immature adults to gamma rays (120-720 r) by whole body irradiation of rat hosts. The frequencies of eight abnormalities occurring in the gravid region were not significantly greater in irradiated worms than those found in controls.

Schiller (1957, 1959) was more successful in altering the morphology of adult Hymenolepis nana by exposing eggs of cysticercoids to unfiltered x-rays (5-40 Kr). He suggested that Palais failed to obtain significant results because she used too little radiation. This is also the probable explanation for Kisner's (1957a) results. Schiller's paper is probably the most significant work published to date on cestode irradiation, since definite induced abnormalities were observed. For the most part, according to Schiller, the observed changes appeared to follow a consistent pattern which could be correlated with the dose of radiation. No new abnormalities were observed, but there was an increase in those exhibited by the species in nature. These results are considered to be especially pertinent to the present study since they are a record of organ level changes which are possibly to be explained at the cellular level.

The most obvious effects observed by Schiller (loc. cit.) of unfiltered x-rays on the morphology of first generation H. nana cysticercoids, irradiated as eggs, were mal-

formations and arrested development. Normal growth and development had been interfered with in three main ways. These were: (1) inhibition of cell division; (2) failure of cells to differentiate; and (3) interference with structural organization. None of the abnormal forms, including those in which development had been arrested, developed to the adult stage when fed to mice. Adults which developed from cysticercoids whose morphology appeared to be normal exhibited a number of structural abnormalities. These were usually found at irregular intervals along the strobila and seldom occurred in a series of adjoining proglottids.

The morphology of adult cestodes after x-irradiation of the cysticercoids was altered to a greater degree than those of worms irradiated as eggs. The anomalous proglottids often occurred together in a series at irregular intervals along the tapeworm body. The testes and ovaries were especially vulnerable. Instead of forming the usual discrete structures, individual cells of these organs were widely distributed in the places where the testes and ovaries were normally located. The cells responsible for the formation of the cirrus and external seminal vesicle appeared to be more resistent, since these organs persisted in proglottids which otherwise manifested the most extreme abnormalities. These observations suggested that cells destined to divide and differentiate into specialized organs were the most

seriously affected by the unfiltered x-rays. Structures which had already attained complete development in the cysticercoid stage before irradiation, such as the scolex, exhibited no observable morphological changes. Kuhlman (1960) also found substantial increases in certain kinds of morphological abnormalities in adult Hymenolepis microstoma after irradiation of the cysticercoids.

Schiller analyzed second generation worms which originated from eggs exposed to 5, 15, and 20 Kr at the beginning of the first generation. According to his tabulations, the proportionate number of proglottids showing a certain type of abnormality (testis-deletion or the absence of one of the three testes) was considerably higher in worms of the second generation than in worms receiving no radiation. The percentage of proglottids affected in this manner was the same, regardless of the original amounts of radiation. When compared with the results obtained by the analysis of this condition in parent worms, it was seen that these percentages were slightly higher than the maximum recorded in specimens from direct infections with eggs irradiated with 25 Kr. Concerning this relationship Schiller made the following statement: "The significance of this apparent relationship is not understood, although there can be little doubt that the testis-deletion effect is, in some way, transferred to the second generation individuals."

Morphological abnormalities are the end products of some interference with the normal pattern of growth and differentiation in organs where they occur. The cells of these structures, during the developmental period, are undergoing rapid cell division. It is well known that cells in this state are most sensitive to ionizing radiations. Hundreds of papers have been published in which specific changes have been discussed (summarized by Dobzhansky, 1951; Hollaender, 1954; Lea, 1955). Schiller's data do not appear to depart from the general pattern of radiation damage reported for many other organisms. It appears that organs damaged most severely where those in which cell division would have occurred after the irradiation. From these data it is established that tapeworm organs can be affected by radiation in sufficient quantity. At this point, it would seem appropriate to determine if induced chromosomal changes in irradiated cestode cells may be a possible explanation for some of the observed abnormalities.

The present chromosome study of H. diminuta grew out of Kisner's (1957a) attempt to affect the morphology of this species with gamma rays. The chromosomes of this worm were known and the number and morphology recorded (Jones, 1945; Kisner, 1957b). In early cleavage stages, the chromosomes are large enough (3-7 microns in length) to observe without difficulty. The diploid number (12) is low enough to enable

the observer to separate and distinguish individual chromosomes. A preliminary examination of embryos from worms receiving too little radiation to induce abnormalities in adult organs revealed what appeared to be some chromosomal aberrations. The question arose as to why there was evidence of cellular damage without detectable morphological change. The present study developed from an attempt to determine the kind and amount of visible cellular damage in irradiated worms. While it was anticipated that such an investigation would not provide the detailed information which has been obtainable with other organisms such as the grasshoppers or Tradescantia, because of the relatively small size of cestode chromosomes, the problem was approached with the intention of obtaining as much information as time and material would permit. The following points were to be studied and analyzed as far as reasonably possible:

1. The normal meiotic and mitotic cycles in oocytes and early embryos respectively.

2. The cleavage pattern in early embryos as it relates to mitosis at this period of development.

3. Confirmation of the actual existence of aberrations in irradiated cells of early embryos.

4. The various types of chromosomal aberrations, if actually induced by radiation.

5. Estimation of the approximate extent of damage

and its correlation with the dose of radiation.

6. Effect of radiation on later generations of worms.

7. Estimation of number of cleavage divisions between irradiation and time of fixation through a study of the growth pattern of developing worms.

MATERIAL AND METHODS

All observations were made on a strain of *Hymenolepis diminuta* obtained through the courtesy of Dr. C. G. Goodchild, Emory University, Georgia. The infection has been maintained in this laboratory since July, 1956. Flour beetles (*Tribolium confusum* and *T. castaneum*) were used as intermediate hosts. Stock cultures of these beetles were kept in the laboratory in containers of vitamin-enriched flour.

The technique for infection of the intermediate hosts, although not one of the conventional methods, was quite effective. Beetle larvae, starved for twenty-four hours, were forced to feed upon infected rat feces for one or two days. The larval beetles were found to be more easily infected than adults, since their greater feeding activities permitted more cestode eggs to be picked up from the fecal material. The intermediate hosts were transferred to flour for fifteen days (at room temperature) to insure the maturation of all developing cysticercoids.

Beetle larvae were dissected in saline, under a dissecting microscope, with the aid of small needles. Cysticercoids were removed from the hemocoel and fed to white rats (Rockland strain) in known numbers (10 per rat). In the early part of the work, cysticercoids were deposited in

the rat esophagus by use of a hypodermic syringe and large-gauge, blunt needle. Later it was considered easier to anesthetize the host animals and infect them via a polyethylene tube. Worms were permitted to reach maturity (18-20 days) in the rat intestine before the irradiation.

Gamma rays were delivered from a cobalt-60 source located at the U. T. AEC Agricultural Research Station, Oak Ridge, Tennessee. Rats were placed in individual plastic containers and set upon a movable platform that revolved around the source. Part I of the chromosome experiments involved the exposure of eight rats (4 males and 4 females) to either 400 or 800 r gamma rays at a dose rate of 8.35 r per minute (24 inches from the source). Worms were fixed at intervals of eight and twenty-four hours after the completion of the radiation. Two additional rats of each sex served as controls. At the second irradiation, twelve male rats all received 1200 r at a dose rate of 3.09 r per minute (48 inches from the source). Worms were fixed at intervals of 2, 3, 4, 5, 6, 7, 8, 10, 12, and 14 hours after completion of the irradiation. For a large dose of radiation, one rat infected with twenty cysticercoids was exposed to 5000 r gamma rays (84 r per minute at a distance of 9 cm. from the source). These worms were fixed eight hours after the end of the irradiation. To determine something about the survival and transmission of possible chromosomal aberrations

to later generations of H. diminuta, an irradiated "strain" was produced. Mature cysticercoids in beetles were exposed to 15000 r. The embryos of adults of the various irradiated generations were to be examined for chromosomal aberrations.

All rats were killed with a blow on the head. Worms were removed from the intestine, washed in 0.85 per cent saline, and blotted quickly with filter paper. They were fixed in Carnoy's fluid (6:3:1 alcohol-chloroform-acetic acid). After twelve hours in the fixative, the tapeworms were removed to 70 per cent alcohol for storage. The only slide preparation in which chromosomes could be successfully studied was a temporary squash stained with aceto-orcein. Other dyes either stained too lightly or were not selective enough. Worms stored in 70 per cent alcohol became progressively harder and more difficult to squash. Moreover, after several weeks stainability was so poor that this material had to be discarded. Later it was discovered that hardening and loss of stainability could be prevented if worms were stored in the fixative in the refrigerator. They remained usable for as long as two months.

Aceto-orcein squashes (Darlington and LaCour, 1960) were made from portions of the strobila which contained embryos in early cleavage stages. Tiny fragments (each approximately one-half proglottid) were mounted in the stain. Great pressure on the cover-glass was required to

flatten the embryos sufficiently for easy observation. A quick seal of the squash preparation was effected by ringing the cover-glass with clear fingernail polish. Slides were stored in the refrigerator. The change between room temperature and that of the refrigerator often produced air leaks which subsequently ruined the slide. These air leaks may be prevented by recoating the original seal with Permount (a commercial preparation obtainable from General Biological Supply House, Chicago, Illinois). When Permount is used, it should be permitted to dry before storage of the slide in the refrigerator. All attempts to make permanent mounts failed since the necessary dehydration through an alcohol series reduced chromosome size. Detailed study of chromosomes in dehydrated squashes was not possible. This was also true with sectioned material. Paraffin sections stained with Heidenhain's iron hematoxylin were unsatisfactory for this material. It was excellent for differentiating chromosomes, but also stained a number of very coarse granules in the embryonic cells. The stained granules prevented observation of chromosomes in most embryos. Embryos were observed with an oil immersion objective (95 X) using the 20 X ocular. All drawings were made with the aid of a camera lucida using the 20 X ocular. A few photographs of normal metaphase plate and seven of the more extreme aberrations are included in the appendix for comparison with the

illustrations.

A growth study similar to that of Chandler (1939) was conducted. If a correlation between a certain amount of growth (linear increase) and a small time interval could be established, it might be possible to determine the approximate stage of an embryo at the time it was irradiated. There is no method, at present, to determine how many cell divisions have occurred between the time these embryos were irradiated and the time of fixation. The number of cell divisions which could occur might be ascertained by counting the cleavage divisions between the region of meiosis and some point along the strobila separated by a distance equal to the linear increase per given time interval. Rats were fed ten cysticercoids and killed in groups of four each day for a period of twenty-one days. Worms were flushed out of the intestine, relaxed in the refrigerator, and fixed in hot alcohol-formalin-acetic acid solution. All cestodes were measured to determine the amount of daily linear increase.

OBSERVATIONS

I. NORMAL DEVELOPMENT

Meiosis

The normal meiotic and mitotic cycles in germ cells and embryos were determined from observation of thousands of dividing cells. The proposed patterns are illustrated in the appendix, but a brief description of each will be included in this section. Cells in the desired states of division were found in the posterior part of the anterior third of each worm. As this species is protandrous, maturation of the testes occurs before that of the ovaries. Chromosomes of the testes are quite small (Figs. 17 and 18). Little was learned from testis material other than a confirmation of the correct diploid number (from diakinesis in primary spermatocytes). It was also possible to determine size relationships of chromosomes in a complement.

As the ovaries mature, they become lobed and packed with germ cells. The germ cells undergo a mitotic multiplication which could not be clearly seen in aceto-orcein squashes. It is not possible to flatten the ovaries sufficiently at this stage for easy observation of chromosomes. After the period of multiplication and just prior to their passage from the ovary to the tubular uterus, the oogonia have a very characteristic appearance (Figs. 12 and 21).

They are spherical or oval although the shape may be grossly distorted by the squashing process. The nucleus is very large in proportion to total cell volume. A single large nucleolus is present. The staining reaction of the nucleus is very distinctive. In fresh squashes, the nucleus appears to have little affinity for the stain. A chromatin "network", characteristic of most stained interphase cells, is not observable. The nucleoplasm is somewhat clear and non-granular as compared to a finely granular cytoplasm. With reduced light and careful focusing, the nuclear boundary may be seen. In sectioned oogonia stained with Heidenhain's iron hematoxylin, delicate chromatin threads may be observed in the nucleus.

Oogonia pass from the ovary to the uterus. The details of this process have not been observed because of spatial disturbances arising from the squashing. By the time the oogonia have reached the uterus, a number of changes has occurred. The first of these is an increase in size. This is accompanied by the attachment of a small cell (Figs. 19 and 20) to each primary oocyte, as the oogonium is now called. The origin of this cell is unknown, but its distinctive nucleus resembles that of cells in the vitelline gland, which is situated immediately posterior to the ovary in intact proglottids. This cell, for purposes of future reference, will be referred to as the "attached" cell.

Chromosomes of the primary oocyte become faintly stainable and begin to shorten and thicken. The early phases of prophase (leptotene through diplotene) could not be clearly observed because of poor affinity of the chromosomes for the dye. By late prophase (diakinesis) the chromosomes become more stainable and assume an irregular, fuzzy appearance (Fig. 22). The nuclear membrane and nucleolus disappear soon after.

Late prophase chromosomes undergo a marked contraction and are deeply stainable (Figs. 23 and 24). Six thick, heavy bivalents are formed and become arranged on a portion of the spindle approximately mid-way between the poles. There is no great degree of regularity about this orientation. Ordinarily the spindle is not visible in the squash preparation. It may be seen in oocytes stored for a time in 70 per cent alcohol. It is usually formed at right angles to the cell surface next to the "attached" cell. The volume of the spindle in proportion to cell volume is much greater than in some organisms such as Ascaris.

Dyads separate and move to the poles at anaphase I (Fig. 25). The first polar body forms to one side of the position of the "attached" cell (Figs. 25 and 26). It is a very compact mass of nuclear material surrounded by a thin layer of cytoplasm. It is approximately the same size as the sperm which at this time is located in the cytoplasm of

the oocyte (Fig. 26). The sperm entered this cell some time prior to metaphase I. It is always present in the cytoplasm by the time bivalents are attached to the spindle.

The dyads remaining in the oocyte (Fig. 26) do not pass through a telophase stage, but immediately become associated with the spindle for metaphase II. Monads separate in anaphase II and the second polar body is formed at the side of the "attached" cell opposite to the position of polar body I. Apparently the spindle rotates somewhat away from that position occupied during the first meiotic division. The monads remaining in the oocyte pass through a telophase and a nuclear membrane is formed (Figs. 13, 14, and 28). The egg nucleus then becomes a typical interphase nucleus. During the second meiotic division, the sperm nucleus becomes active. It increases in size and loses its stainability as the nucleus goes into interphase (Fig. 28). The final result is a male pronucleus which is approximately the same size as the egg nucleus. The male and female pronuclei do not fuse with each other in the formation of the zygote. Each enters a typical prophase separately. Chromosomes begin to condense and in favorable material, six long chromatin threads may be counted in each pronucleus (Fig. 29). The pronuclear membranes disappear and fully contracted chromosomes become attached to the first cleavage spindle (Fig 30).

Early Cleavage Divisions

Cleavage divisions I through VI follow a specific pattern as illustrated by Figs. 1, 30-46, and 53-60. The largest cell of the embryo is always involved in each of the six divisions. Each of the first five divisions is unequal, producing a large and a small daughter cell. The smaller cells, after they are formed, do not divide in the early embryo. The large cell of the embryo is several times larger than any other present as far as the six cell stage. The staining reaction of its nucleus is very weak, similar to that of an oogonium. Unless the cell is dividing, the nucleus is practically invisible. Such cells are usually represented in illustrations of the appendix by the outlines of their cell membranes. Smaller cells may be identified by their nuclear sizes. The cell membranes of the smaller cells do not stain, and only their nuclei are included in the illustrations in most instances. The small cell of cleavage I is several times larger than those formed by the next three divisions. Small cells of divisions II through IV are approximately the same size and have not been distinguished from each other when all are present in the same embryo. It should be noted that all small cells are cut off from the larger one in the vicinity of the "attached" cell, which may be recognized by its thick, heavy chromatin net-

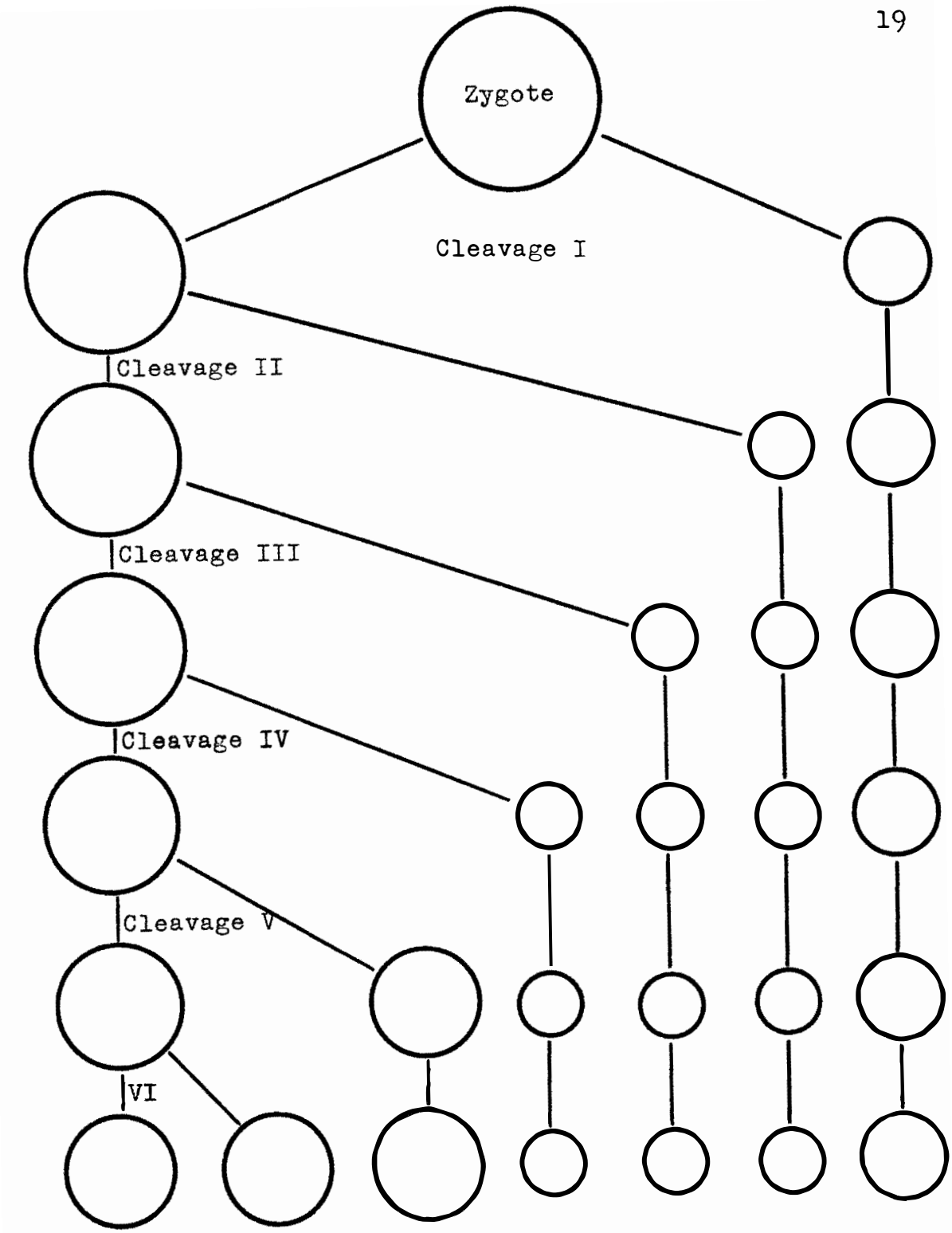

Figure 1. Proposed cleavage pattern for Hymenolepis diminuta in cleavages I-VI.

work. The products of cleavage V are somewhat different from those of previous divisions. The smaller cell of this division is a little larger than the small cell of the first cleavage. Cleavage VI divides the largest embryonic cell into two parts which are more or less equal in size. The cleavage pattern of subsequent divisions was not studied, although illustrations of embryos with as many as eight or nine cells are included in the appendix (Figs. 51 and 52). In the seven cell embryo, mitosis was sometimes observed to occur in two of the larger cells at the same time (Fig. 50). Perhaps the cleavage pattern changes somewhat after the seven cell stage.

Chromosomes

The diploid chromosome number of Hymenolepis diminuta was determined by Jones (1945) and Kisner (1957a). There are six pairs as seen in metaphase plates of dividing embryonic cells (Fig. 30). There are six bivalents in diakinesis of testis cells (Figs. 17 and 18). A similar number is found in metaphase I of primary oocytes (Figs. 22 and 24). All chromosomes are acrocentric. Separation in early anaphase always begins at one end of the metaphase chromosomes (Fig. 45). In anaphase, all chromosomes are rod-shaped as expected if centromeres are terminal or nearly so (Figs. 31 and 46). Kisner (1957b) measured chromosomes in metaphase

plates of seventeen cells of embryos considered to be in cleavage I. Average sizes of the six pairs were 7, 5.5, 5, 5, 4.5, and 3 microns (for idiogram see Fig. 16). Metaphase chromosomes of the largest embryonic cell of the first few cleavages are approximately the maximum size, but as the size of this cell decreases through the mitotic subdivision, the chromosomes also become smaller. Any actual measured size must be regarded as only approximate because of the staining and mounting technique employed. The dye as applied is dissolved in 45 per cent acetic acid, which tends to soften and swell tissues. The absolute size of cells and chromosomes may also change slightly with time. Also, some cells may have originally been flattened to a greater degree than others through greater pressure on the cover-glass when the squash was made. For the above reason, in the illustrations, the absolute size of cells in one embryo should not be compared with the absolute size of like cells of other embryos.

II. RADIATION EFFECTS

Thousands of dividing cells from unirradiated worms were observed during the course of the study. The only abnormal condition ever observed was the occasional presence of a cell with one more or one less chromosome than the diploid number. In irradiated worms, however, chromo-

somes in embryonic cells exhibited many aberrations. For purposes of description, the latter have been classified according to effect instead of the amount of radiation received or the time between irradiation and fixation. This has been necessary since some effects were observed at all doses and times of fixation studied. To describe all effects in each situation would involve needless repetition. The usable technique for worm storage was not discovered until late in the experiments. Many preserved worms had to be discarded because of the hardening effect of the alcohol. Some worms from rats receiving all mentioned doses were successfully studied, but the fixation times should be mentioned. At 400 r, worms were observed at eight hours. For the 800 r dose, worms were used that had been fixed at eight and twenty-four hours after irradiation. At 1200 r, fixation times were two and seven hours, while all worms receiving 5000 r were fixed at eight hours after rats were irradiated.

In the appendix, there are a number of illustrations in which the cleavage division was undetermined. During the early part of the observations, some irradiated embryos were observed before the correct cleavage pattern was understood. When a striking aberration was found, the entire embryo was drawn without regard to the cleavage division. After the cleavage pattern was determined, the temporary squash pre-

parations in which the aberrations had been found were no longer usable. Determination of the cleavage division of these was made from the illustration instead of the embryo itself. In all cases, the probable cleavage division has been indicated. Each was included because it was a good example of some aberration. The uncertainty as to the correct division is not important for the immediate purpose intended.

Chromosome "Stickiness"

Aberrations resembling the effect referred to as "stickiness" (Lea, 1955) were observed in worms receiving 1200 r. Most cells affected in this manner were found in embryos fixed two hours after irradiation. A few were noted in embryos fixed at seven hours. In affected cells, chromatin appeared as deeply stained masses or clumps (Fig 94). Sometimes, individual chromosomes could not be distinguished. A certain proportion of the damaged cells exhibited both chromatin clumps and chromosomes, as well as a number of fragments scattered in the cytoplasm (Figs. 95 and 96). At anaphase, long chromatin bridges were present (Figs. 84, 85, and 94). A few appeared to possess most of the chromatin material of the cell and were extremely thick and heavy. Bridges were of unequal diameter along the nuclear strand (Figs. 84 and 85). They appeared to be pulled out into thin

strands at some points which intervened between the thicker portions.

Chromosome Bridges

Unlike the "sticky" bridges just described, the chromosome bridges referred to here are products of chromosome and chromatid breaks. This type was observed in irradiated embryos at all doses and fixation times studied, but were more abundant in those fixed at eight hours. The number per affected cell ranged from one to as many as three, with one being the most common number. There were two kinds, if classified on the basis of thickness of the nuclear strand. One type was thick and heavy (Figs. 81 and 83) while the other kind was approximately half the diameter of the first (Fig. 89). Some extended the entire distance between the poles. When the length of the bridge exceeded this distance, a bend or slight coiling of the chromosomal strand was noted (Figs. 8 and 81). Other bridges were apparently much shorter than the distance between the poles. Such bridges were often broken with the break being observed at any point along the chromosomal strand (Figs. 90 and 91). An unequal breakage of the bridge would lead to one cell with a chromosome having a portion deleted and another with a duplication. Fragments were usually associated with the bridges. They were found to be scattered at any point in

the cytoplasm of the damaged cell (Figs. 81 and 83).

Changes in Chromosome Number and Fragmentation

These two types of aberrations are here considered together. It is practically impossible to distinguish the smaller chromosomes of this tapeworm from fragments of the same size except by position in the cell. A chromosome will usually have a particular orientation during any one of the mitotic phases. A fragment without a centromere may not always have this orientation. When a chromosome was far removed from others of the cell, it was considered to be a fragment. Fragmentation was evident in all irradiated material observed. It occurred separately and in combination with all other aberrations mentioned. The number of cells with fragments was greater in worms from rats receiving the higher doses. There was evidence of mass fragmentation at 5000 r (Figs. 115 and 118). The chromosomes of some cells were nothing more than small bits of chromatin. In a few cells, there was an interesting situation in which chromosomal elements existed in the cytoplasm of cells in interphase (Figs 107 and 108).

Increased chromosome numbers were more frequent than decreased numbers, ranging from 2n minus seven to 2n plus twenty-seven. The increase did not follow any consistent pattern such as an increase by haploid sets or doubling.

Cells with thirteen, fourteen, and fifteen chromosomal elements were most common. Some of the increases were undoubtedly a result of fragmentation. From the standpoint of time involved, it was not worthwhile to attempt to distinguish the latter from true increases in chromosome number. In the sample count, (Table I) they were lumped together.

Long Chromosomes

The most interesting of all aberrations was the occurrence of chromosomes whose lengths exceeded the normal for the species. These were found in embryos of worms from rats receiving all mentioned doses and fixed at seven, eight, and twenty-four hours after the end of the radiation period. The frequency of this aberration was never very great. The sample count at 5000 r (Table I) shows that only twelve embryos in 990 observed were affected in this manner. Although few were found when the total was considered, the aberration was not a rare one derived from a single worm or worms from a single rat. One could expect to find it in any of the above mentioned material.

There was no single length which was characteristic for the long chromosomes. Some were only slightly longer than the longest chromosome of a normal complement. Others were as many as five or six times longer than any other in

the cell. Examples of the shorter ones may be seen in Figs. 63-65. Figs. 66-74 show chromosomes at the other extreme. One of the clearest examples of the aberration is illustrated in Fig. 70 and the photograph (Fig. 5). There are only twelve chromosomes in the cell, including the long one. Chromosome fragments, typical of most of the abnormal cells, are absent. The orientation of one end of the long chromosome suggests that a centromere may be present. Fig. 71 is an illustration of the chromosomes of Fig. 70 ranked in order of size. Almost all of the twelve chromosomes of this cell may be grouped in pairs, except for the long one which obviously lacks a homolog. The excess length definitely presents a mechanical problem at anaphase when daughter chromosomes are separating. The length of a few greatly exceeded the entire distance between the poles. Various configurations of coiling and bending showed how this problem was temporarily solved (Figs. 8, 9, and 81). Sometimes the centromere ends of the chromosomes had already reached the poles while the other double ends remained at the equatorial plane, still unseparated. Figs. 7 and 77 illustrate one long chromosome coiled in the cytoplasm and slightly removed from others of the cell. It is assumed that a centromere is not present on this one. Just how the long chromosomes finally complete the anaphase separation and

become incorporated into daughter nuclei has not been observed.

Other Aberrations

Although the above mentioned aberrations comprised the major types observed, a few others were found occasionally or perhaps only once. Obvious dicentric chromosomes were observed only once (Fig. 10). In this embryo, two U-shaped chromosomes are approaching one pole of the cell with another of the same type near the other pole. Chromosome "rings" were found in one embryo (Fig. 104). Four small doughnut-shaped chromosome rings are located in the cytoplasm near the metaphase plate. Aberrations of meiotic chromosomes were not studied because of their small size. A few abnormalities were observed and one is included (Fig. 97). Six dyads are present in meiosis II plus an extra monad which lacks a mate. Fig. 98 is an illustration of a primary oocyte with two of the "attached" cells instead of the usual one. Embryos of Figs. 97 and 98 were found in irradiated embryos, but the abnormalities may not be products of radiation damage since they are occasionally observed in any worm, either irradiated or unirradiated.

Frequency of Aberrations

After a confirmation of the existence of chromosome

aberrations in irradiated embryos, an attempt was made to determine something about the frequency at which they occurred. It was found not to be very practical to make large scale counts of embryos. Beck (1951) found an average of 2100 eggs per gravid proglottid in worms from singly infected rats on a normal diet. Of course, an immature tapeworm would not contain this many developing embryos, but there would be thousands in the usable portion of each worm. Much time would be required to accumulate data on large numbers of worms. Another problem was the mixture of different stages found in a single proglottid. In a young proglottid, most of the cells may be in the meiotic stages. As the proglottid grows older, zygotes undergo cleavage while the ovary is still producing germ cells. In some of the older proglottids there was a range of stages from oocytes to embryos containing the largest numbers of cells studied. The tabulation of results would be much simplified if all cells and embryos in a single proglottid were in the same stage. In general, it may be stated that only a few cells per hundred were affected at a dose of radiation as low as 400 r. There was an obvious increase at 800 and 1200 r, with the maximum observed at 5000 r. Sample counts of damaged embryos were made from three worms receiving the latter dose. Totals of each type of aberration for the var-

ious cleavage divisions are presented in Table I. There were 451 embryos with altered chromosomes in 990 observed, or 45.5 per cent. The per cents for each cleavage division (I through V) are included in Table I. A histogram of these per cents is found in Fig 2. On the basis of per cent affected, it appears that there was an increase of damaged embryos in each division from cleavage I through cleavage III, with an almost constant per cent for all cleavage divisions III through V. A chi square test was completed on the assumption (theoretical expectation) that each stage would have the same per cent of aberrations as the whole sample showed. It was concluded that there was a significant difference among the percentages of abnormalities in different cleavage divisions.

Inheritance of Radiation Effects

Ten cysticercoids receiving 15000 r were fed to a rat. Four adult worms were recovered from this rat when surviving cysticercoids reached maturity. These worms were fixed for chromosome study after enough eggs had been collected to produce a second generation of cysticercoids. The second generation of cysticercoids received an additional 15000 r. Approximately fifty were introduced into a rat. Some of the cysticercoids reached maturity, for a few eggs were observed in the fecal material of the host. A third

TABLE I

SAMPLE COUNT OF NORMAL AND DAMAGED MITOSES IN EMBRYOS OF THREE WORMS* FROM A RAT RECEIVING 5000 r GAMMA RAYS

Type of Damage	Cleavage division examined				
	I	II	III	IV	V
Increased chromosome number or fragmentation	92	56	97	78	60
Decreased chromosome number	4	4	3	4	3
Chromosome bridges	6	4	7	6	15
Long chromosomes	0	2	5	3	2
Total number of damaged mitoses	102	66	112	91	80
Total number of mitoses observed	304	151	210	172	153
Per cent damaged mitoses	33.55	43.70	53.33	52.90	52.21

*Fixed eight hours after irradiation.

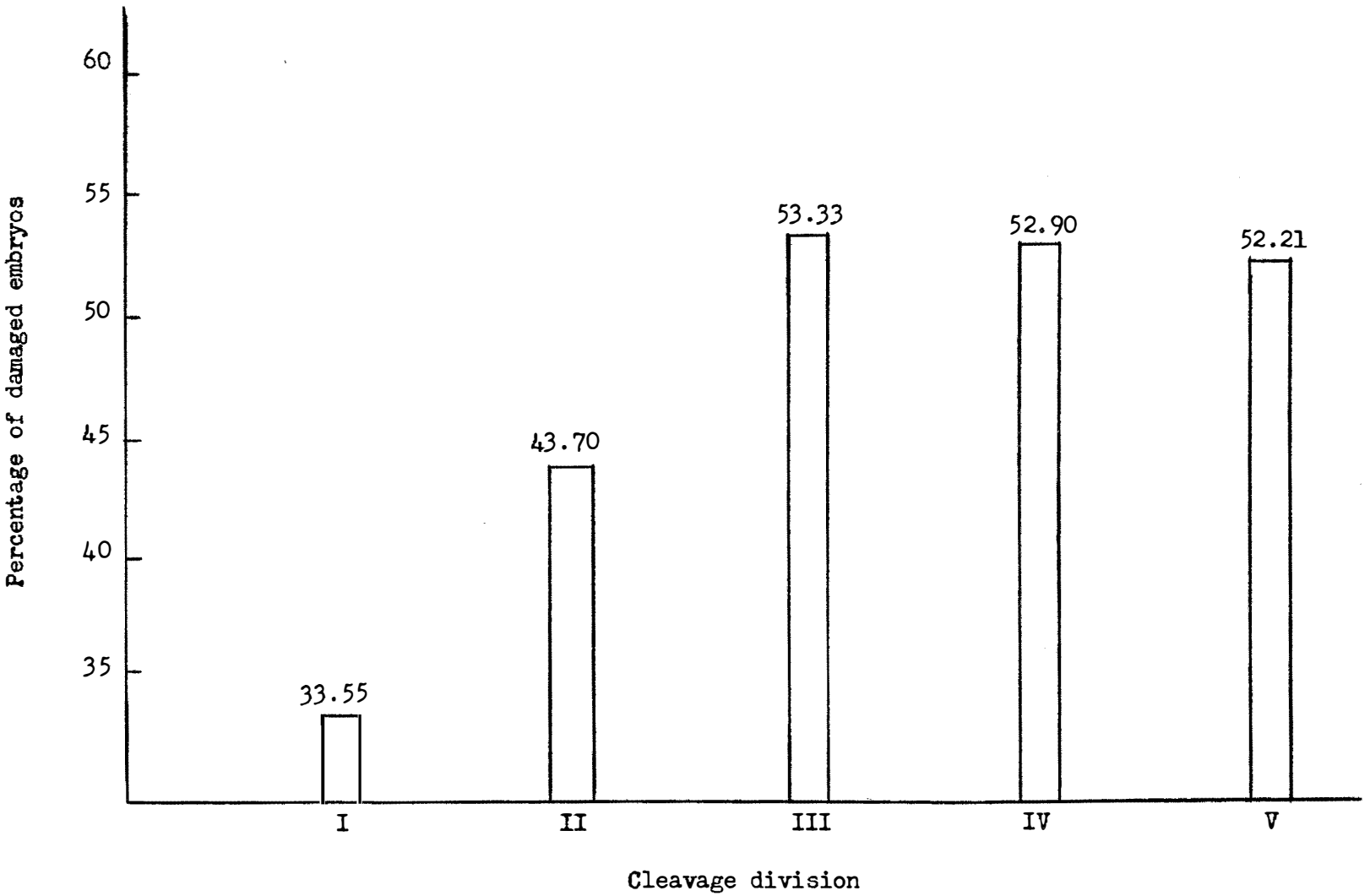

Figure 2. Percentages of embryos damaged in five cleavage divisions as determined from three worms in rats receiving 5000 r gamma rays.

generation of cysticercoids was produced, but the experiments were terminated by this time, and a third generation of adults was not obtained by the author. Moreover, there was not sufficient time to examine the chromosomes in embryos of the first generation adults. This part of the experiments was continued by others in this laboratory.

III. GROWTH RATE STUDY

The growth rate study was intended to provide information as to the number of cleavage divisions possible in any given length of worm in the region containing embryos. It was unsuccessful for the purpose intended because the infection rate was very low. The experiment was set up so that a sample of four rats, each infested with ten worms, could be killed daily for twenty-one days. From the measurement of these worms, a growth curve was to be established similar to that of Chandler (1939). If worms could be expected to grow a certain amount per given time interval, one could count the number of cleavage divisions in a measured portion containing embryos, and determine the total number possible for that specific part. This could be compared with the number of cleavages found in a portion of the strobila of the same length in an irradiated worm. The number of worms recovered per rat was quite inadequate to determine a reliable growth curve.

In considering the data collected, an unexpected variability was observed. While it does not deal directly with chromosomes, brief mention of this particular observation should indicate perhaps that it would not be possible to predict a number of cleavage divisions in a particular length of worm containing embryos, that would be applicable to other worms. The histograms of Fig. 3 show that worm lengths varied within rats, and between rats which were comparable in weight, sex, age, worm burden, and genetic background.

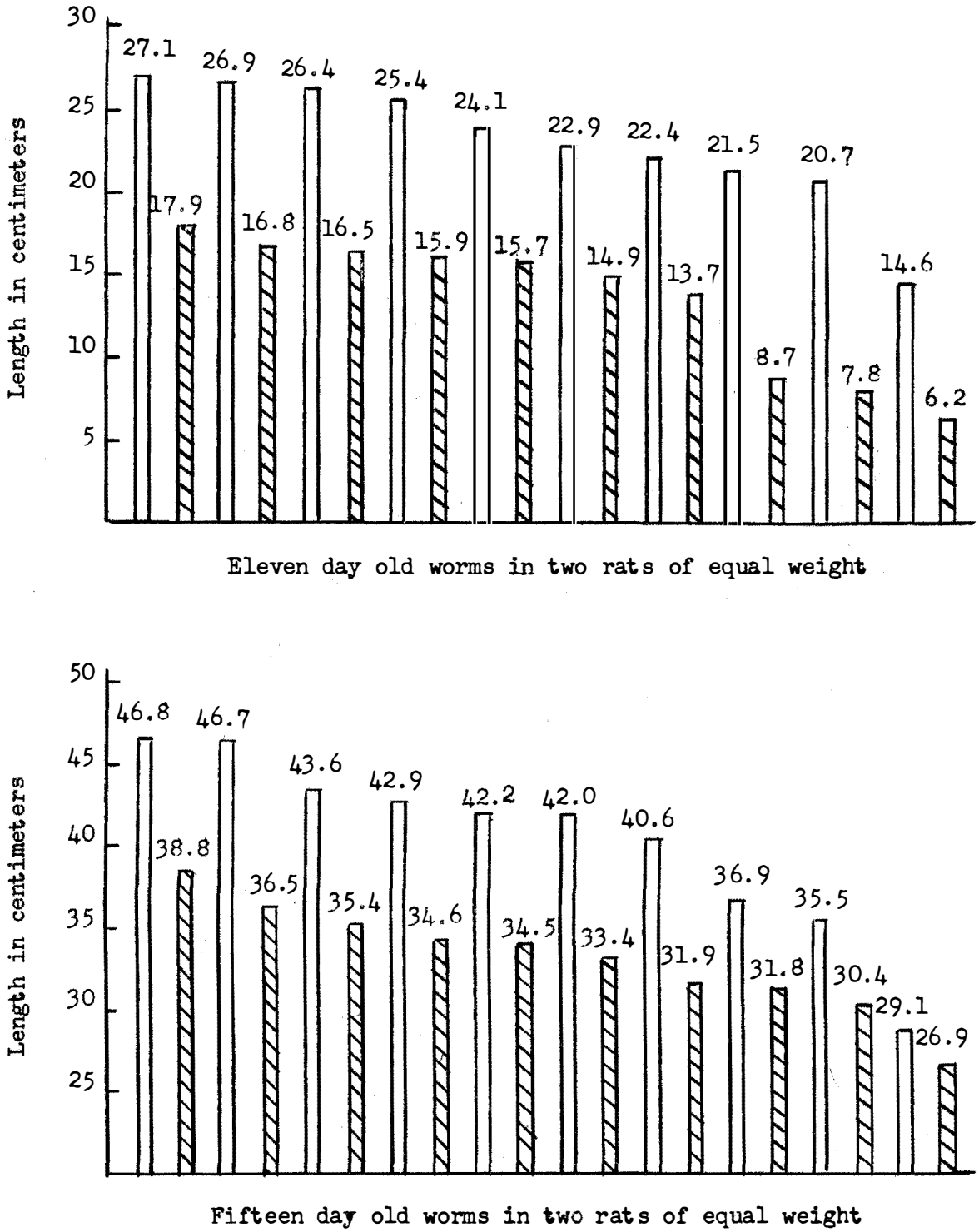

Figure 3. Comparison of lengths of eleven and fifteen day old worms.

DISCUSSION

The chief objective of the present study has been to determine if and how cestode chromosomes might be affected by radiation. It was, of course, necessary to determine the normal condition in regard to the embryos and their chromosomes. Before the cleavage pattern was understood, the cells, nuclei, and chromosomes of different sizes were quite confusing. When dividing cells in various embryos were observed, it was not known if they were of the same or different stages. The squashing process only added to the confusion by eliminating size as a useful basis for comparison of cells. It was discovered that the corresponding cell in two different embryos might be quite unlike in size because of the squashing process. It was essential that an understanding of the cleavage pattern precede the study and interpretation of abnormalities.

The cleavage pattern as proposed is unusual. It is known that the amount and distribution of yolk has an effect upon the cleavage of an egg. The zygote of H. diminuta contains yolk which may be considered in regard to the effect it might have upon the cleavage pattern. The large granules mentioned as having been found in the cytoplasm of oocytes and large cells of embryos are assumed to be yolk material. They stain with Heidenhain's iron hematoxylin technique, but

remain uncolored in fresh aceto-orcein preparations. As the squash becomes older, it appears that some of the orcein leaves solution and is deposited around the granules, making their outlines visible. The granules are always found in the large cells through the embryonic stages studied. Few, if any, are found in the small cells produced by divisions I through IV. Apparently some factor, perhaps yolk, prevents the initial equal division of the zygote. It is subdivided by the "cutting-off" of small cells from the main mass until cleavage VI, when an equal division occurs for the first time in the embryo. The reduction in size of a very large cell by this method would be understandable in terms of yolk present, were it not for the specific manner in which it occurs in H. diminuta. The small cell of cleavage I is always larger than those produced by the next three divisions, and smaller than that formed by the fifth cleavage. Why should a larger cell be "cut off" before smaller ones, if yolk material were the only determining factor in the way the zygote is subdivided? Moreover, cells are added to the early embryo singly instead of by twos, fours, etc., as in Ascaris, the starfish, or the frog.

The proposed pattern of cleavage for H. diminuta (Figs. 1 and 53-60) was compared with Ogren's (1953) proposed pattern for the order Cyclophyllidea. Ogren correlated his observations of cleavage divisions in cestodes

with the spiral type exhibited by many invertebrates. In the proposed pattern into which H. diminuta should fit, the zygote should divide into two macromeres of equal size. In H. diminuta, two cells of quite unequal size result from cleavage I. Ogren further states that in the second cleavage division, one of the macromeres from cleavage I divides again equally, producing two more macromeres. The author has found that cleavage II in this species produces two cells that would be referred to as a macromere and a micromere, if Ogren's terminology were employed. In the present paper, the terms large cell and small cell have been used to mean macromere and micromere respectively. In the third cleavage, according to Ogren's scheme, a macromere divides unequally, producing a macromere and a micromere. This was found to be true in H. diminuta, but the cell which divides to form them is not the same as that indicated by Ogren's text figure of the division. Ogren indicated that the micromere divides in cleavage IV to form two more micromeres. The author has not observed micromeres in states of division in any of the first six cleavages of this species. Cleavages V and VI of Ogren's plan show a macromere dividing to produce another macromere and a micromere. This does not correspond to cleavage V in H. diminuta, where the largest cell of the embryo divides to form another large cell and one which is approximately half the size of the latter.

This smaller cell is larger than cells previously referred to as micromeres. And in cleavage VI, instead of a macromere and a micromere resulting, there is an approximately equal division of the largest cell in the embryo.

Ogren's alternative plan also, in certain respects, does not correspond with that observed in this species. Cleavage I is again presented as an equal division; and in the fourth cleavage, it is indicated that a micromere divides, instead of one of the larger cells. It is unclear, at present, why the above-mentioned discrepancies should exist. It might be stated that Ogren's observations were made chiefly from stained whole embryos and sections. His observations were not cytological in nature. His conclusions, however, were based on data from a number of representatives of the Cyclophyllidea, although H. diminuta was not one of them. H. nana, a related species in the same genus, was included in his study, and should be similar to H. diminuta in many respects. The present study was mainly cytological, and Ogren's techniques were not employed. The latter method would be necessary for an adequate comparison of the cleavage patterns considered.

The term "attached" cell is used merely for the identification of a particular cell. It is suggested that the origin of the cell is the vitelline gland because of similarities of staining reaction. This origin cannot be deter-

mined with certainty from squashes, and should be verified from sectioned material. The function of this cell, if any, is unknown in the early embryo. It remains with the embryo, without visible change, through all the early stages observed. As mentioned previously, polar bodies and small cells are always found in the vicinity of the "attached" cell. Perhaps this cell has some influence in determining just where the small cells will form.

Data accumulated for radiation effects studied do not differ greatly from those reported for other irradiated organisms. The "sticky" chromosomes were observed soon after irradiation. It is reported (Lea, 1955) that this effect appears in cells completing a division after irradiation in one of the mitotic phases. Their presence in some material fixed at seven hours suggests that a few cells were delayed in this completion for five hours longer than most. The presence of numerous fragments and some translocations is to be expected from simple breakage and rearrangements of chromatids and chromosomes.

Some of the long chromosomes were so unusual that special attention should be given to their possible origin. Chromosomes that exceed normal length may arise through translocations and the end to end fusion of pieces from various chromosomes. But there is an expected limit to the size of a long chromosome formed in this manner. Pieces

from several chromosomes may fuse with each other, but it is not likely that all fragments present in an irradiated cell will do so. One would not expect the length of a chromosome arising through translocations to exceed a maximum of several times that of the largest chromosome of a normal complement. Moreover, a corresponding decrease in the lengths of other chromosomes of the cell would be observed. This, undoubtedly, is an explanation for some of the longer chromosomes observed that did not greatly exceed normal size. It would not explain the origin of a chromosome as large as that illustrated in Figs. 5, 70, and 71. The length of this large chromosome is equal to the combined lengths of the next eight largest chromosomes of the cell. Moreover, eleven other chromosomes are present, in addition to the very long one. If the long chromosome could be explained by translocations, surely the other eleven would show greater irregularities than are observable (Fig. 71).

A more reasonable possibility for the origin of long chromosomes of this nature is the chromatid breakage-fusion-bridge cycle, as presented by Faberge (1958). This cycle results from a single break across one chromatid in prophase. The chromatid may consist of two strands which are broken at the same, or nearly the same, level, the two free ends uniting. Alternatively, it might be considered that the chromatid is "single" at the time of breakage, but that when it

doubles, the terminal element fails to duplicate, thus giving an equivalent sister union. The same result would be obtained if the free ends are capable of rejoining immediately after duplication. Regardless of the way in which the cycle is initiated, a bridge is produced at anaphase consisting of a chromatid which is very nearly symmetrical about the preceding point of fusion. When the bridge breaks, broken ends are again exposed which may repeat the whole process in the next division. The cycle may be continued for many cell generations. If the breakage of the bridge always occurred at its midpoint, a chromosome affected in the manner would not increase in length. But it has been observed that a bridge in this material may break at any point along the chromosomal strand. When breakage is unequal, one cell receives a chromosome somewhat longer than that from which the bridge originated. The other cell receives a shortened portion. A chromosome of considerable length might arise when the cycle is continued for several cell generations with the addition of a small portion in each division. An embryo that illustrates a particular way in which a bridge might possibly break is found in Fig. 79. The long bridge is broken at the position through which the cleavage furrow may have cut during cytokinesis. One cell received a very long part of the bridge, while the smaller cell contains the shortened portion. This process could

have been repeated through several unequal divisions.

With the large number of fragments observed in this irradiated material, it is surprising that more dicentrics of the type shown in Fig. 10 were not present. It seems reasonable that no metacentric chromosomes were observed when the position of the centromeres is considered. In H. diminuta, all centromeres are terminal, or very near the end. For a metacentric to form, there must be a break either across the centromere or the short arm of the chromosome, if one exists. There is much less chance for a break to occur at this point as compared to the many possibilities for breaks in the long arm of the chromosome. Probably metacentrics would be found if enough material were examined.

The histogram (Fig. 2) illustrates the per cent of affected embryos in each cleavage division of the sample count at 5000 r. It shows that the greatest number of embryos with chromosome alterations were in cleavage divisions III, IV, and V. A chi square test indicated a significant difference among percentages shown. Unless the embryos included in the count represent an unusual sample, there must be some explanation for the observed differences. All worms were fixed at eight hours after irradiation. There was no evidence of "stickiness" of chromosomes. It is the opinion of the author that by eight hours, "sticky" chromosomes have recovered and that some cell division has occurred. Just

how many divisions is not known. Probably embryos observed at the time of fixation were in some earlier stage at the time irradiated. The observed embryos in cleavages I and II may have been oocytes in meiosis at the time of irradiation. Perhaps the more severely damaged oocytes did not successfully complete meiosis and fertilization. If only moderately damaged oocytes formed zygotes and proceeded as far as cleavages I and II by the time of observation, this would account for the reduced number of affected embryos at these stages. According to this scheme, embryos observed in cleavages III through V would have been in the pronuclear stage and cleavages I and II at irradiation.

The work of Schiller (1959) and Kuhlman (1960) proves that cestode morphology can be affected by radiation. Their observations include only gross changes, with no detailed information as to the mechanisms involved. Schiller's discussion of the subject includes the following remarks:

> Variations are known to have different causes. Permanent and transmissible variations involving chromosome aberrations (e.g.breaks, inversions, translocations, losses, and polyploidy), gene mutations, or segregation have been referred to as genotypic. Variations which occur in response to the nature of the external environment, or the interactions of both, have been classified as developmental or temporary changes. Difficulties in methodology are involved in arriving at a decision regarding the origin of a variation as a genotypic change in a cestode. Although some cytological studies have been reported for a few species (Child, 1904, 1911, in Moniezia; Harman, 1913, in Taenia; Young, 1908, 1912, 1919, 1935, in Taenidae; Motomura, 1929, in Archigetes; Jones, 1945, in Hymenolepididae and Dilepididae; Jones

> and Ciordia, 1956, in Taenia; and Jones and Wyant, 1957, in Taenia) comparatively little is known about heredity in these organisms. In the present state of knowledge with regard to these worms it is not possible to know with certainty if events such as chromosome or gene mutations occur and how important a contribution they make to the observed cases of variation. The problem is not simple since one must depend upon phenotypic expression to indicate the change in the genotype.

Schiller did not define the term chromosome mutation. Apparently he used it to indicate a chromosome aberration or some change other than a gene mutation. While it may not be possible to know with certainty if gene mutations occur in cestodes, it is possible to know if such events as chromosome mutations (aberrations) are possible merely by observing the chromosomes of an irradiated cestode. The chromosomes in embryos of H. nana can be observed just as easily as those of H. diminuta. Perhaps the former species would be a better one for chromosome study than the latter. It is a smaller worm, a fact which would simplify the squashing process. The diploid number is ten (Jones, 1945), or twelve if one obtains the cytological "race" reported by Jones (1955).

Schiller pointed out that certain aspects of the observed effects of radiation on H. nana suggested some of the changes might have been analogous to the phenomena of radiation-induced gene and chromosome mutations as observed in other organisms. The present report verifies the actual existence of aberrations in irradiated cestodes. It does

not indicate to what extent these aberrations affect future generations or to what extent they may influence the production of morphological abnormalities. Perhaps many of the observed aberrations would have been lethal to cells in which they occurred. It would seem reasonable to assume that some of the small changes could persist without fatal effects. Moreover, if radiation can produce such gross changes as observed, surely there would be numerous changes at the level of the gene. One way in which transmissible aberrations might be detected would be the examination of descendants of irradiated worms. It is the opinion of the author that given enough radiation and generations of worms, the morphology of H. diminuta could be permanently changed through the accumulation of chromosome and gene changes. Any visible, persistent aberrations may be detected by examination of dividing embryos.

The neck region of the tapeworm body contains cells with embryonic properties. These are rapidly-dividing cells since new proglottids are constantly being budded off behind this zone of growth. Chromosomes of these cells are very small and study would be difficult, if at all possible. Nevertheless, these chromosomes should be affected in the same manner as those of the embryos. In the sample count (Table I), an average of 45.5 per cent of the embryos exhibited chromosome changes. If a like number of cells in the

neck region were affected, surely there would be some obvious change in the organs which develop from them. It is quite clear why the worms observed by Kisner (1957a) did not show significant radiation induced morphological abnormalities. Immature worms were subjected to the same treatment as those of the present report, but the maximum dose was only 720 r. In irradiated worms at 800 r, large numbers of damaged embryos were not observed. Apparently the damage produced by 720 r in the first experiment was not sufficient to bring about observable changes in the organs. A point that is not clear in connection with the radiation-induced morphological abnormalities reported by Schiller is the way they appear along the strobila. Structural abnormalities in adult worms irradiated as eggs occurred at irregular intervals along the body of the tapeworm. Those in cestodes irradiated as cysticercoids were found many times in series at various intervals along the strobila. If the abnormalities are to be explained by chromosome and gene changes in the cells which form them, why do normal and abnormal proglottids sometimes occur next to each other in a single worm? Both the abnormal and normal proglottid descended from the cells of the neck region. Unless the change occurs after the proglottids are budded off from this zone, it would seem that all would be affected in the same manner.

The present experiments have been a general survey of

cytological effects of radiation on early embryonic stages of H. diminuta. With the major problem of technique already solved, future work could be designed to provide more specific information. This includes further study of effects mentioned in this report. Moreover, the effect of dose rate might be considered. It would be interesting to pursue further the possible origin of the long chromosome aberration. If actually formed in the manner suggested, perhaps some indirect evidence of it could be obtained to support this theory. If several cell generations are required to produce a long chromosome of the type of Fig. 70, one would not expect to find this degree of the aberration soon after irradiation. But as the time between irradiation and fixation increases, there should be a noticeable increase in the average length of any long chromosomes that do appear. If one should find an extremely long chromosome in a worm where most dividing cells exhibit the "sticky" effect, this might lead to some doubt as to the validity of the breakage-fusion-bridge cycle as an explanation.

SUMMARY

Chromosomes of the adult stage of the cestode Hymenolepis diminuta were studied in aceto-orcein squashes of selected portions of the strobila. The details of oogenesis were observed and recorded. They are very similar to those reported for other organisms. Oogonia increase in size and pass to the uterus where meiosis occurs. A small cell of unknown origin and function attaches itself to the primary oocyte. Reduction divisions proceed in a typical manner. Penetration of the oocyte by the sperm occurs prior to metaphase I. Male and female pronuclei do not fuse with each other, but remain separate during prophase. Their chromosomes mingle on the first cleavage spindle at metaphase.

The cleavage pattern during the first six embryonic divisions was determined as a prerequisite to the understanding of mitosis in embryos. The first five cleavage divisions of the embryo are quite unequal. The zygote appears to be subdivided by the "cutting off" of small cells from the main mass during these five divisions. An equal division occurs for the first time at cleavage VI and involves the largest cell of the embryo, as did the first five. The small cells formed during the early cleavages are of different sizes, depending upon the number of the division. The smaller cell formed by cleavage I is larger than

those produced by the next three cell divisions of the embryo. The small cells, after they are formed, do not divide in the early embryo. There are twelve acrocentric chromosomes in metaphase plates. They are of maximum size during the first few cleavage divisions, but become smaller as the large cell is reduced in size by the mitotic subdivision. The mitotic process is very precise with few, if any, irregularities in thousands of divisions.

Mature worms were irradiated with gamma rays (400, 800, 1200, and 5000 r) by whole-body irradiation of rat hosts. Fixation times were 2, 7, 8, and 24 hours after irradiation. As the dose of radiation increased, the number of embryos with aberrations also increased. "Sticky" chromosomes were found in worms examined soon after irradiation. Fragments and bridges appeared in all material observed. Variations of chromosome number, above and below the diploid number, were noted. Chromosomes exceeding normal length were seen in a few cells. Moderately long ones were probably a result of translocations. The extremely long ones suggested a chromatid breakage-fusion-bridge cycle. A sample count of aberrations in the first five cleavage divisions was made in three worms receiving 5000 r. There were 451 embryos with chromosome alterations in 990 observed, or 45.5 per cent of the total. A greater percentage of these was found in cleavages III through V than was observed in

the first two divisions.

A growth rate study for H. diminuta in ten worm infestations was attempted. The desired number of worms was not recovered from enough rats to determine a reliable growth curve. It was discovered that worm length varied within rats and between rats of comparable sex, age, weight, worm burden, and genetic background.

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APPENDIX

PLATE I

EXPLANATION OF FIGURES

Figures 4--10 are photographs of embryos prepared by aceto-orcein squash method. No magnifications are given. Camera lucida drawings of Figures 4, 5, 6, 7, and 11 may be found in the illustrations (with correct dimension scale). The reader is referred to these.

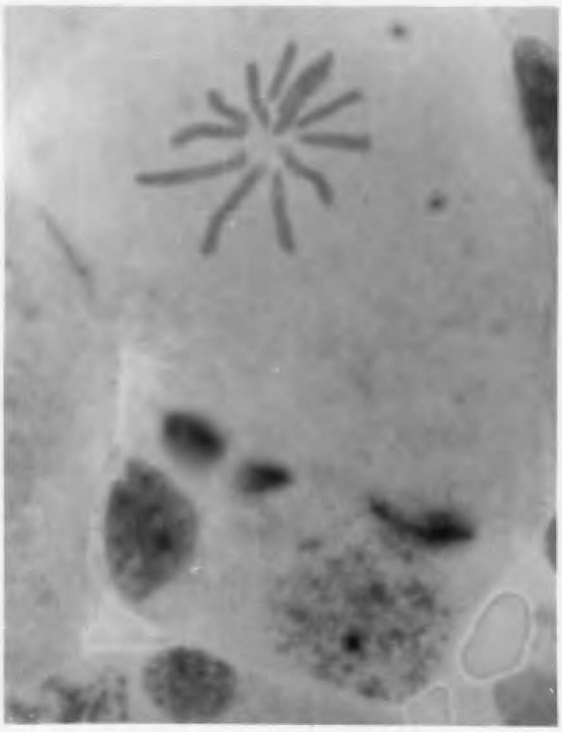
Figure 4. Cleavage V. Polar view of a normal metaphase. The faint outline of the largest cell is visible. Cell membranes and cytoplasm of the smaller cells are not visible while their nuclei are quite distinct. The largest nucleus (lower right of embryo) belongs to the smaller cell of cleavage I. The dark staining nucleus next in order of size (lower left of embryo) is part of the "attached" cell. The three small nuclei indicate cells which are products of cleavages II, III, and IV. See Fig. 42.

Figure 5. Cleavage stage undetermined. Probably cleavage V. An extreme example of the long chromosome abnormality is shown. Only twelve chromosomes (the diploid number) are present. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after end-point of irradiation.

Figure 6. Cleavage stage undetermined. Probably cleavage V. Example of the long chromosome abnormality.

The largest nucleus present belongs to the smaller cell arising from cleavage I. The nucleus of the "attached" cell is next in order of size. The three small nuclei of approximately equal size are those of the smaller cells of cleavages II, III, and IV. The small black masses are very likely polar bodies. Chromosomes are of the metaphase degree of thickening. Twenty chromosomal elements are present in addition to a very long one. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-point of irradiation. See Fig. 67.

Figure 7. Cleavage V. Twenty-six chromosomal elements are distributed unequally between the poles. One very long chromosome, apparently acentric, is coiled in the cytoplasm. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-point of irradiation. See Fig. 77.



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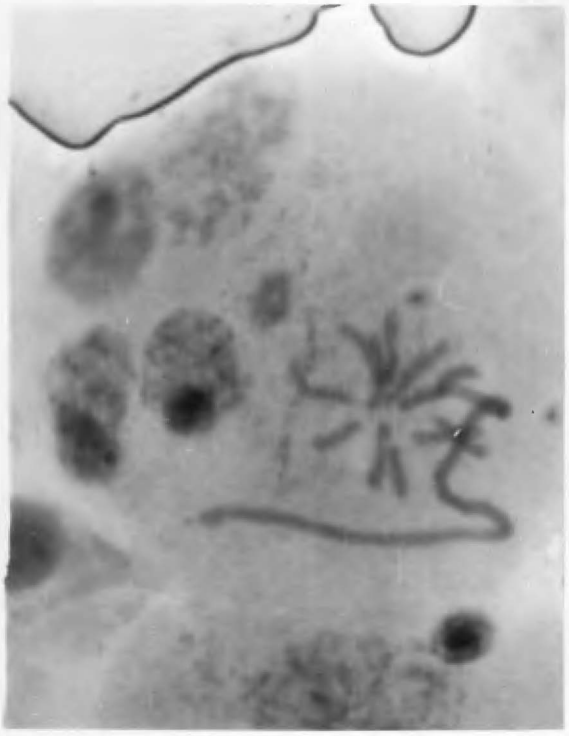

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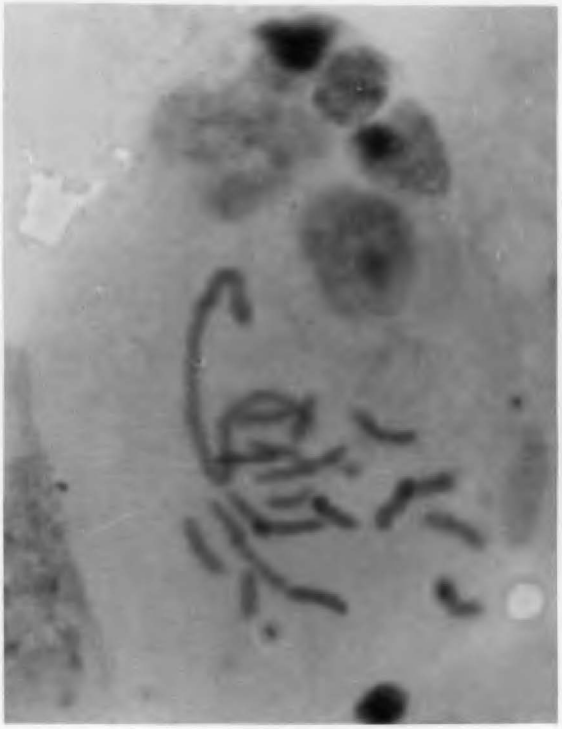

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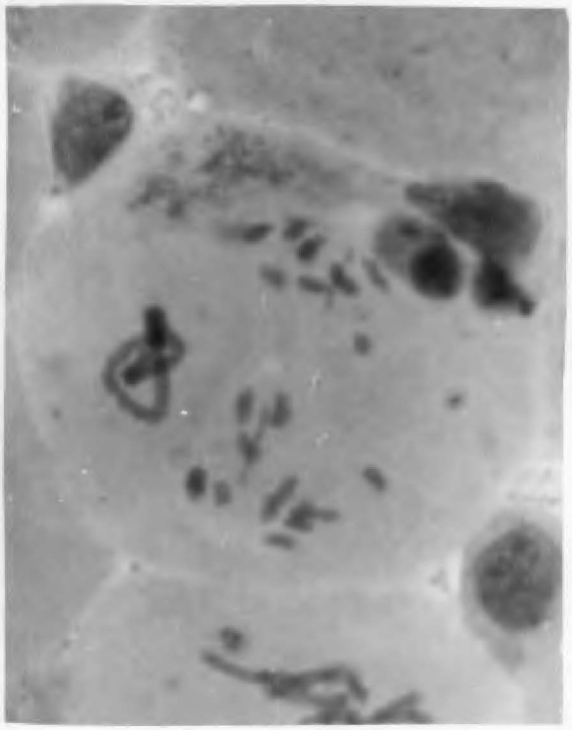

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PLATE I

PLATE I continued

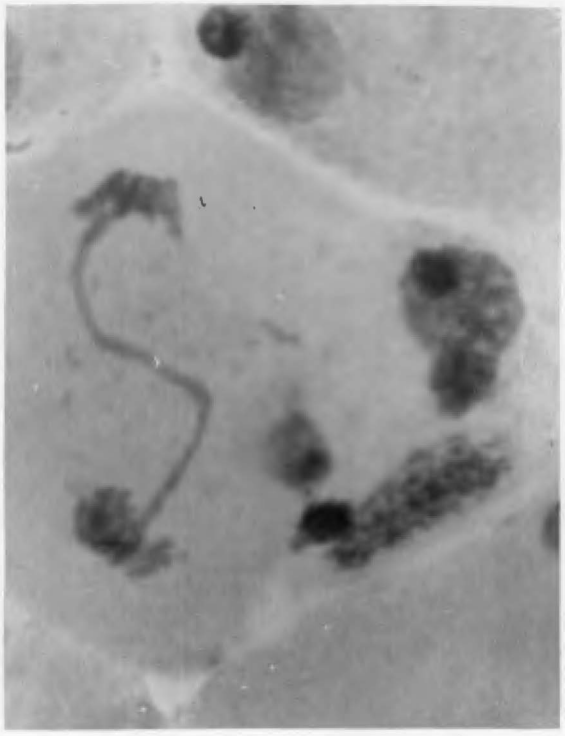
EXPLANATION OF FIGURES

Figure 8. Cleavage stage undetermined. Probably cleavage V. Late anaphase or early telophase. Long bridge is bent in the region of the equatorial plane. 800 r at 8.35 r per minute. Worm fixed at approximately eight hours after the end-point of irradiation.

Figure 9. Cleavage stage undetermined. Probably cleavage V. Late anaphase or early telophase. Long bridge bent in region of the equatorial plane. Fragments in the cytoplasm. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-point of irradiation.

Figure 10. Cleavage stage undetermined. Probably cleavage IV. Anaphase. Three U-shaped chromosomes approaching the poles are assumed to be dicentrics. 800 r at 8.35 per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 11. Cleavage V. Metaphase. This is an example of an increase in chromosomal elements. It would appear from the orientation of most of them that centromeres are present. 800 r at 8.35 r per minute. Worm fixed eight hours after the end-point of the irradiation. See Fig. 103.



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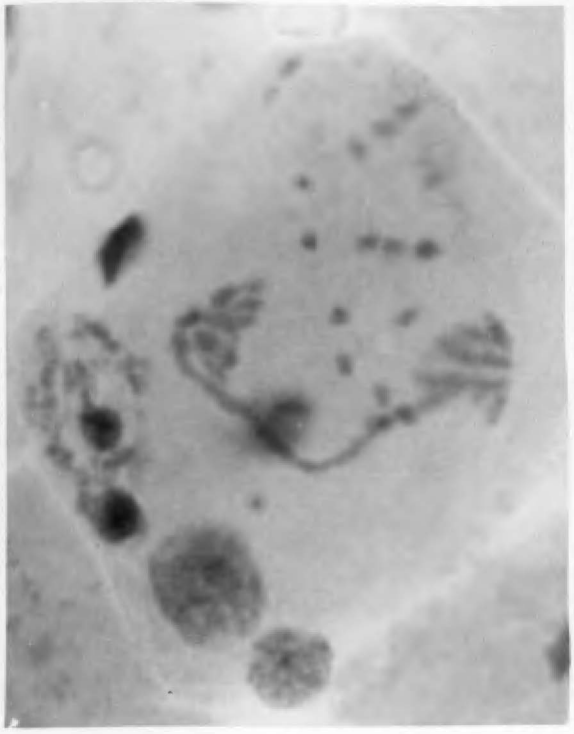

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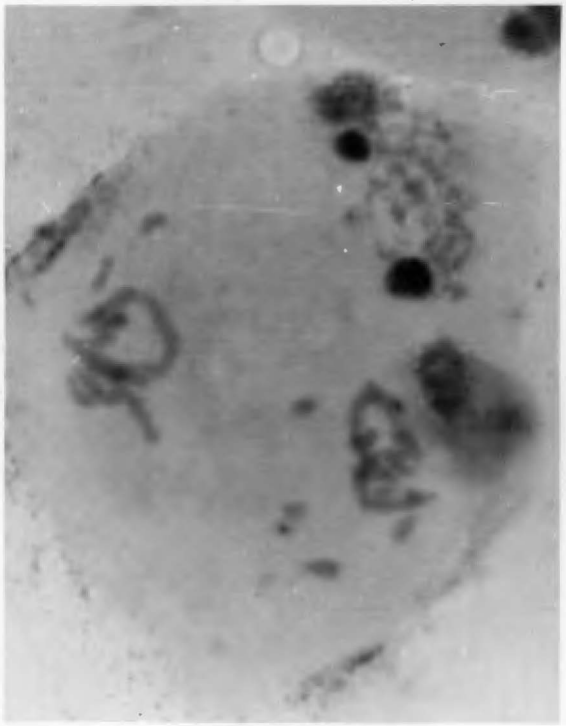

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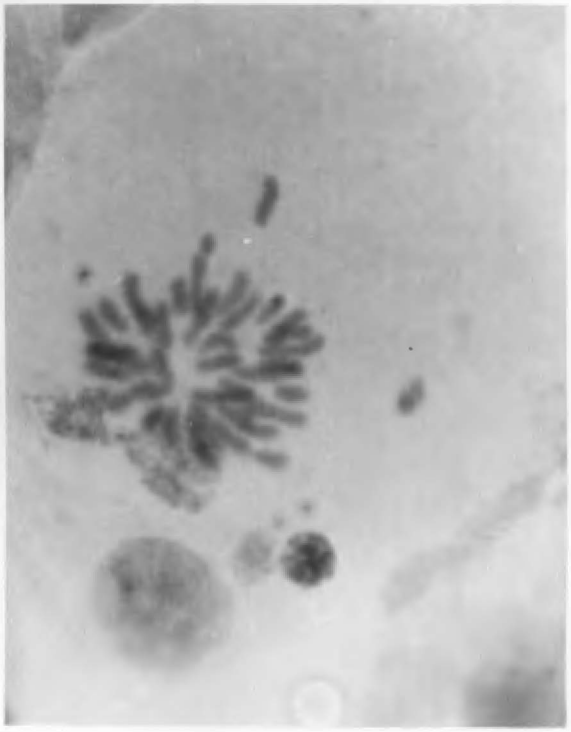

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PLATE I continued

PLATE II

EXPLANATION OF FIGURES

Figures 12--62 represent normal material. Figures 21--28 illustrate meiosis. Figures 29--62 present mitosis and the evidence upon which the proposed cleavage pattern was based. All illustrations were drawn with the aid of a camera lucida using an oil immersion objective (95X) and a 20 X ocular. Figures 12--15 are from sectioned material (10 microns) and stained with Heidenhain's iron hematoxylin (H.I.H.). All others were drawn from aceto-orcein preparations.

Figure 12. Oogonium as it appears in the ovary prior to the beginning of meiosis. A large nucleus and nucleolus are present. Many very coarse dark-staining granules are found in the cytoplasm.

Figure 13. Pronuclear stage. Pronuclei are in interphase. Each possesses a nucleolus.

Figure 14. Same as Fig. 13. Note the presence of a larger number of granules in the cytoplasm.

Figure 15. Cleavage in embryo. Polar metaphase. Stage undetermined. Note the presence of twelve chromosomes which are all acrocentric.

Figure 16. Idiogram of metaphase chromosomes. Cleavage I. Determined from seventeen cells.

Figure 17. Diakinesis in testis cell.

Figure 18. Same as Fig. 17

Figure 19. A cell that attaches to the primary oocyte. It remains with the embryo during early cleavage, apparently unchanged. This cell resembles those of the vitelline gland.

Figure 20. Same as Fig. 19.

Figure 21. Oogonium as it appears in the ovary prior to the beginning of meiosis. See Fig. 12.

Figure 22. Very early meiosis in the primary oocyte. Chromosomes possessing irregular outlines appear in groups of two before the disappearance of the nuclear membrane or nucleolus. The small nucleus containing a thick irregular chromatin network belongs to the "attached" cell.

Figure 23. Meiosis. Metaphase I. Note that fertilization has already occurred. The sperm nucleus is the compact spherical mass in the cytoplasm.

Figure 24. Meiosis. Side view of metaphase I. Outline of spindle is shown. Note that it occupies much of the interior of the cell. The large masses outlined in the cytoplasm are granules swollen by acetic acid.

Figure 25. Meiosis. Anaphase I. Note position of future polar body in relation to the "attached" cell.

Figure 26. Meiosis. Metaphase II. The smaller of

the compact masses in the cytoplasm is the sperm nucleus; the larger is polar body I.

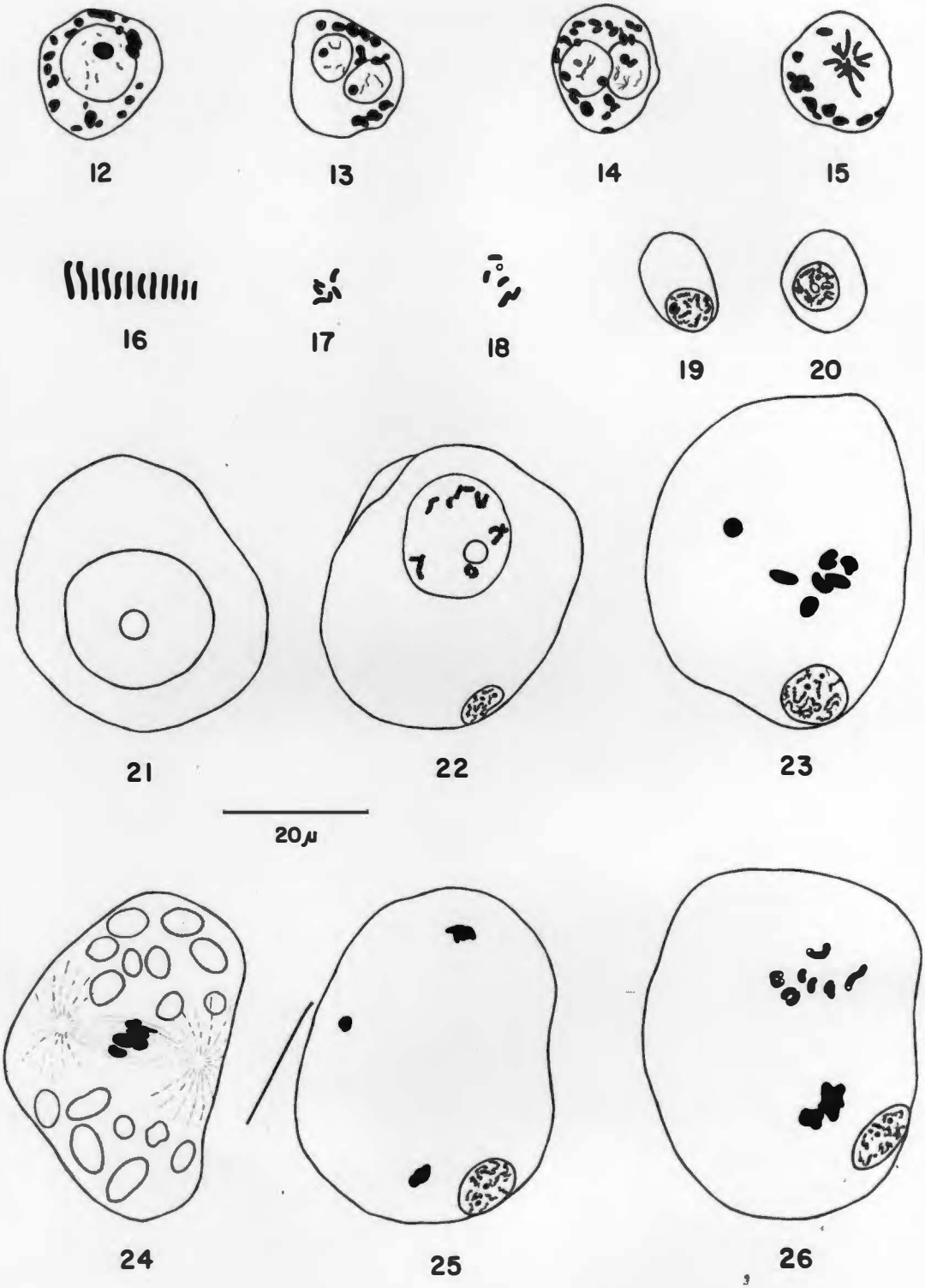

PLATE II

PLATE II continued

EXPLANATION OF FIGURES

Figure 27. Pronuclear stage. Pronuclei do not stain well with aceto-orcein. The two polar bodies are located near to the "attached" cell. Outlined masses in cytoplasm are those of Figs. 12--15.

Figure 28. Outlines of male and female pronuclei are visible. Outermost membrane of the cell is the vitelline membrane where it has become detached.

Figure 29. Cleavage I. Prometaphase. The chromosomes of each pronucleus are condensing. Pronuclear membranes are no longer present.

Figure 30. Cleavage I. Polar view of metaphase. "Attached" cell is absent.

Figure 31. Cleavage I. Late anaphase. The chromosomes are approaching the poles of the cell, but still maintain individual distinctness. "Attached" cell is absent.

Figure 32. Cleavage I. Telophase. Cleavage furrow is beginning to form. Note unequal division.

Figure 33. Two cell stage. Division of the zygote has produced a very large cell and a small one.

Figure 34. Cleavage II. Polar view of metaphase. Sizes of cleavage II chromosomes are approximately the same as those of cleavage I.

Figure 35. Three cell stage. Nucleus of very large cell has not stained. The nucleus of the smaller cell resulting from cleavage II is not as large as that of the smaller cell of cleavage I.

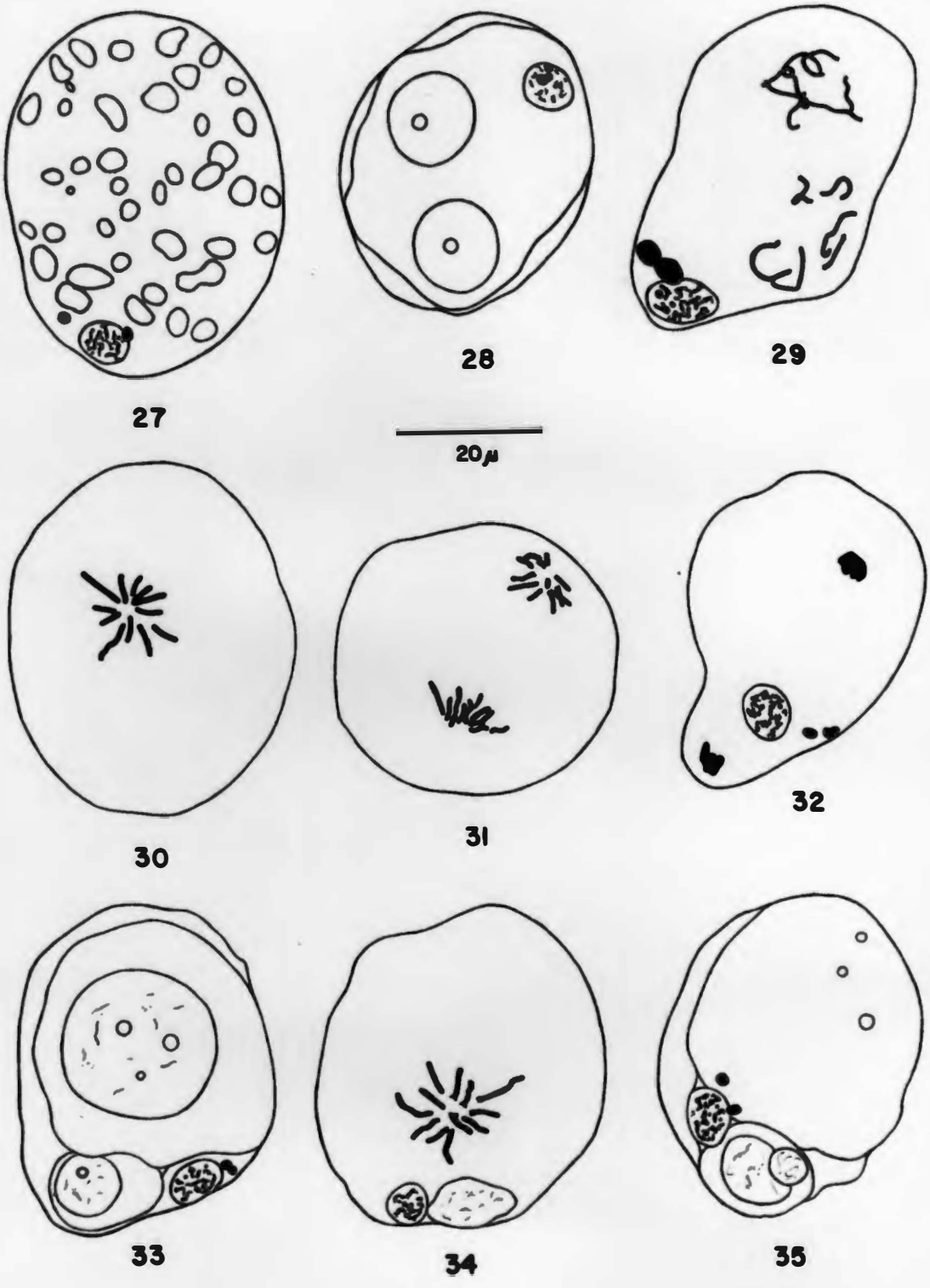

PLATE II continued

PLATE II continued

EXPLANATION OF FIGURES

Figure 36. Four cell stage. The nucleus of the smaller cell of cleavage III is approximately the same size as the smaller nucleus resulting from cleavage II. Nucleus of largest cell is not stained.

Figure 37. Cleavage IV. Prometaphase. Chromosomes are contracted and deeply stainable but not yet oriented on the metaphase plate. Note that mitosis is occurring in the largest cell of the embryo and not in one of the micromeres.

Figure 38. Cleavage IV. Telophase. The nucleus of this division and those of previous divisions appear to be located near the "attached" cell.

Figure 39. Five cell stage. The cell membranes of the three smaller cells were not visible in the squash. Only their nuclei are drawn.

Figure 40. Same as Fig. 39. Cell membranes of the smaller cells were not visible while the nucleus of the large cell could not be seen.

Figure 41. Cleavage V. Prometaphase. Chromosomes not fully contracted nor arranged on a metaphase plate.

Figure 42. Cleavage V. Polar view of metaphase. See Fig. 4.

Figure 43. Six cell stage. Note that the smaller

cell produced by cleavage I.

Figure 44. Same as Fig. 43. Note large cell without a visible nucleus in lower left of embryo. This cell was added by cleavage V.

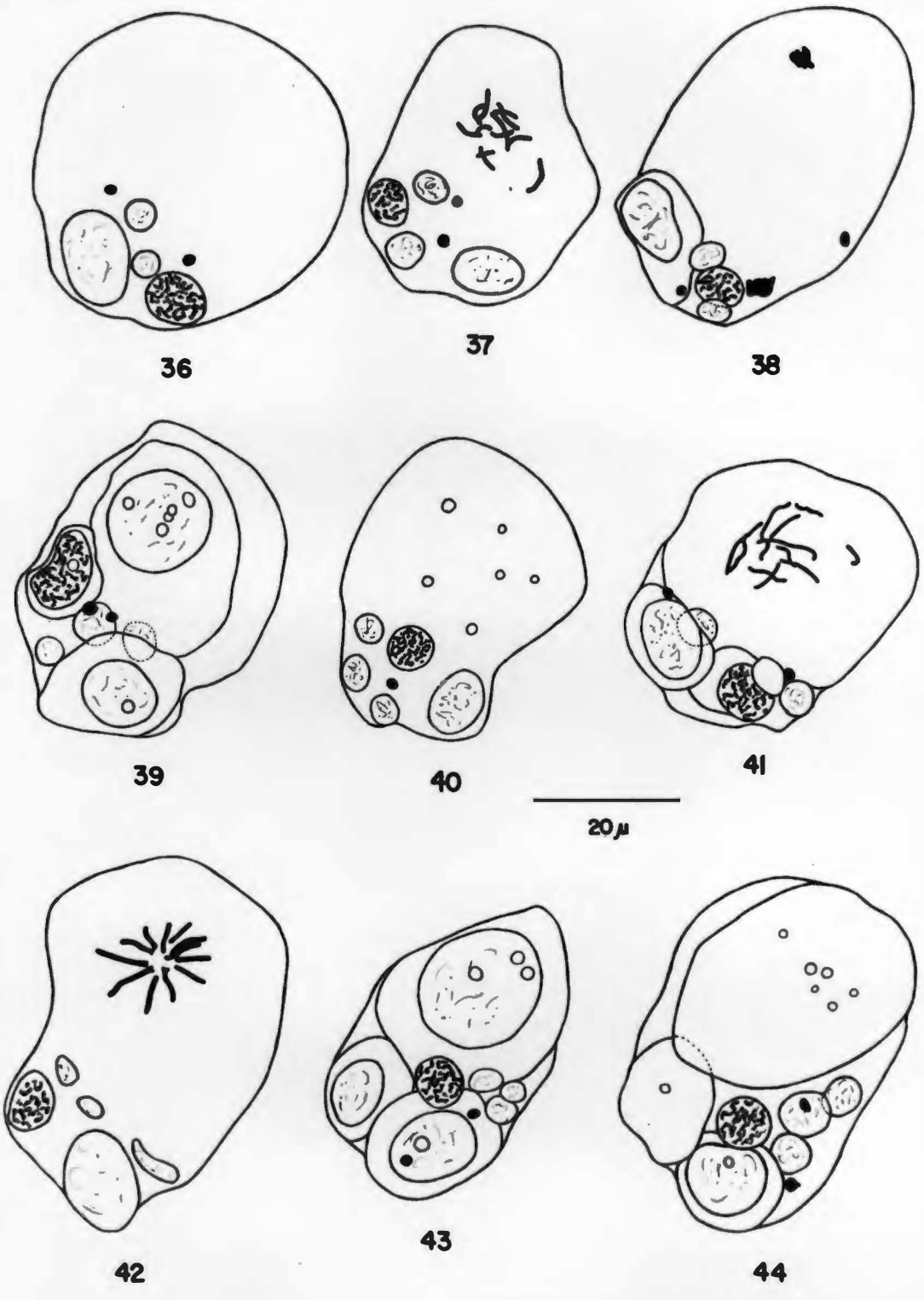

PLATE II continued

PLATE II continued

EXPLANATION OF FIGURES

Figure 45. Cleavage VI. Early anaphase. Note that each of the metaphase type chromosomes are separating at the ends, indicating the position of the centromeres.

Figure 46. Cleavage VI. Late anaphase. Chromosomes are still distinct. All are rod-shaped in anaphase.

Figure 47. Seven cell stage. The largest cell of the six cell stage has divided almost equally.

Figure 48. Same as Fig. 47. Large cells on the right of the embryo are products of cleavage VI.

Figure 49. Same as Fig. 47.

Figure 50. Seven cell stage. Mitosis occurring in two of the largest cells. This may indicate that the cleavage pattern changes somewhat after the seven cell stage.

Figure 51. Eight cell stage.

Figure 52. Nine cell stage. Note that the polar bodies are still present in the embryo.

Figures 53--62 are included to show specifically the cleavage pattern in the early embryonic stages studied. The stage of mitosis in each dividing cell is telophase.

Figure 53. Cleavage I. Telophase. The cleavage furrow is visible. Note that the chromosomes which will be included in the smaller cell appear to be more advanced in

telophase than those of the large cell.

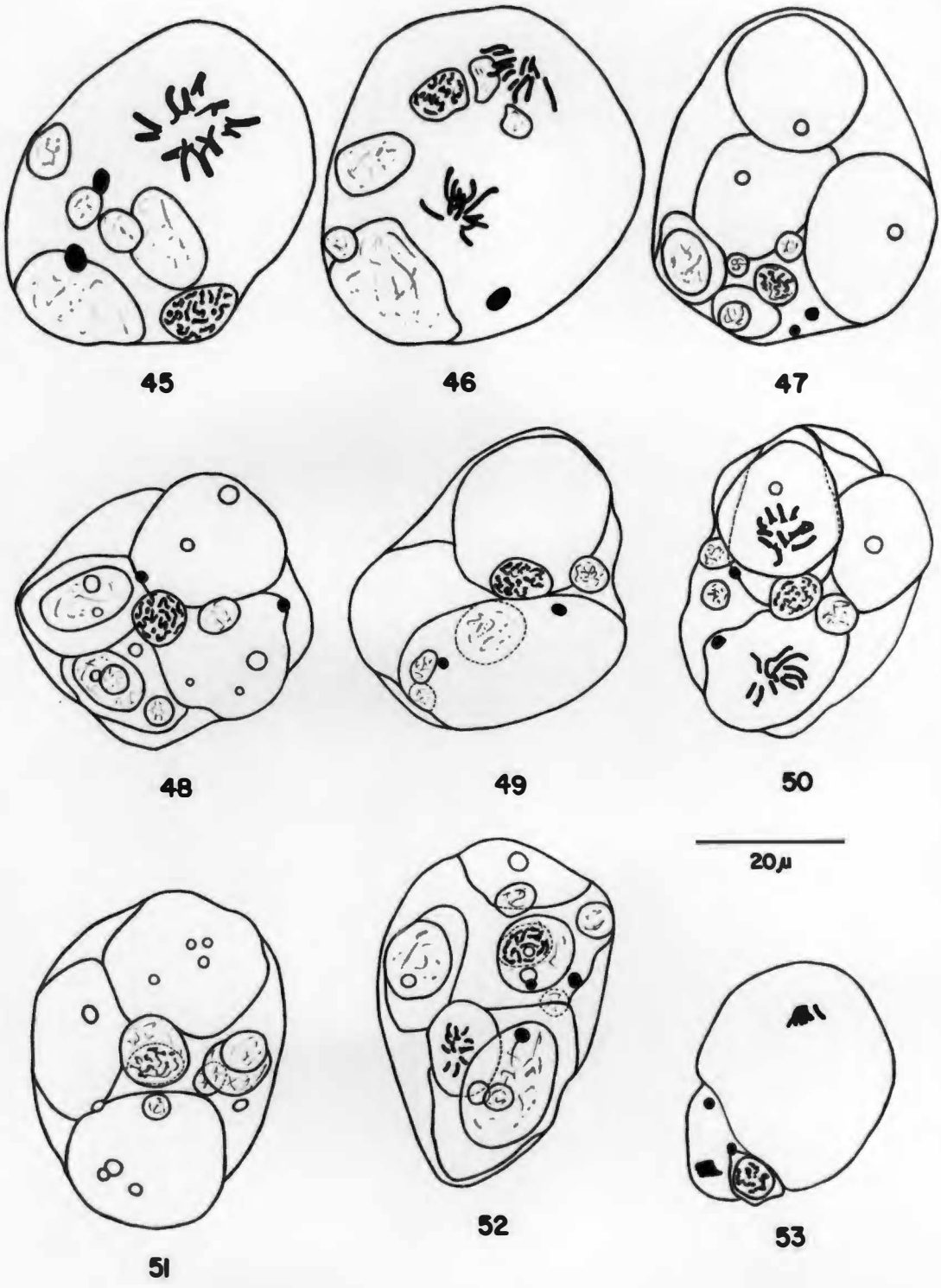

PLATE II continued

PLATE II continued

EXPLANATION OF FIGURES

Figure 54. Cleavage II. Telophase. The result of this division will be a very large cell and a very small one.

Figure 55. Cleavage III. Telophase. Note that again a small cell will be cut off from the largest cell of the embryo.

Figure 56. Cleavage IV. Telophase. A small cell is in the process of being cut off from the largest cell of the embryo. The cleavage furrow has not yet formed.

Figure 57. Cleavage IV. Telophase. The cleavage furrow has formed and the products of the fourth cleavage division clearly demonstrated.

Figure 58. Cleavage V. Telophase. The largest cell of the embryo is beginning to undergo a type of division which is almost equal.

Figure 59. Cleavage V. The cleavage furrow has formed and cytokinesis is nearly complete.

Figure 60. Cleavage VI. The largest cell of the embryo is dividing equally.

Figure 61. Cleavage VII. Telophase in a large cell. It is not known which cell divides beyond cleavage VI. Possibly more than one may divide as shown in Fig. 50.

Figure 62. Cleavage VIII. Telophase in one of the

larger cells of the embryo. Note particularly the polar bodies with a small amount of cytoplasm.

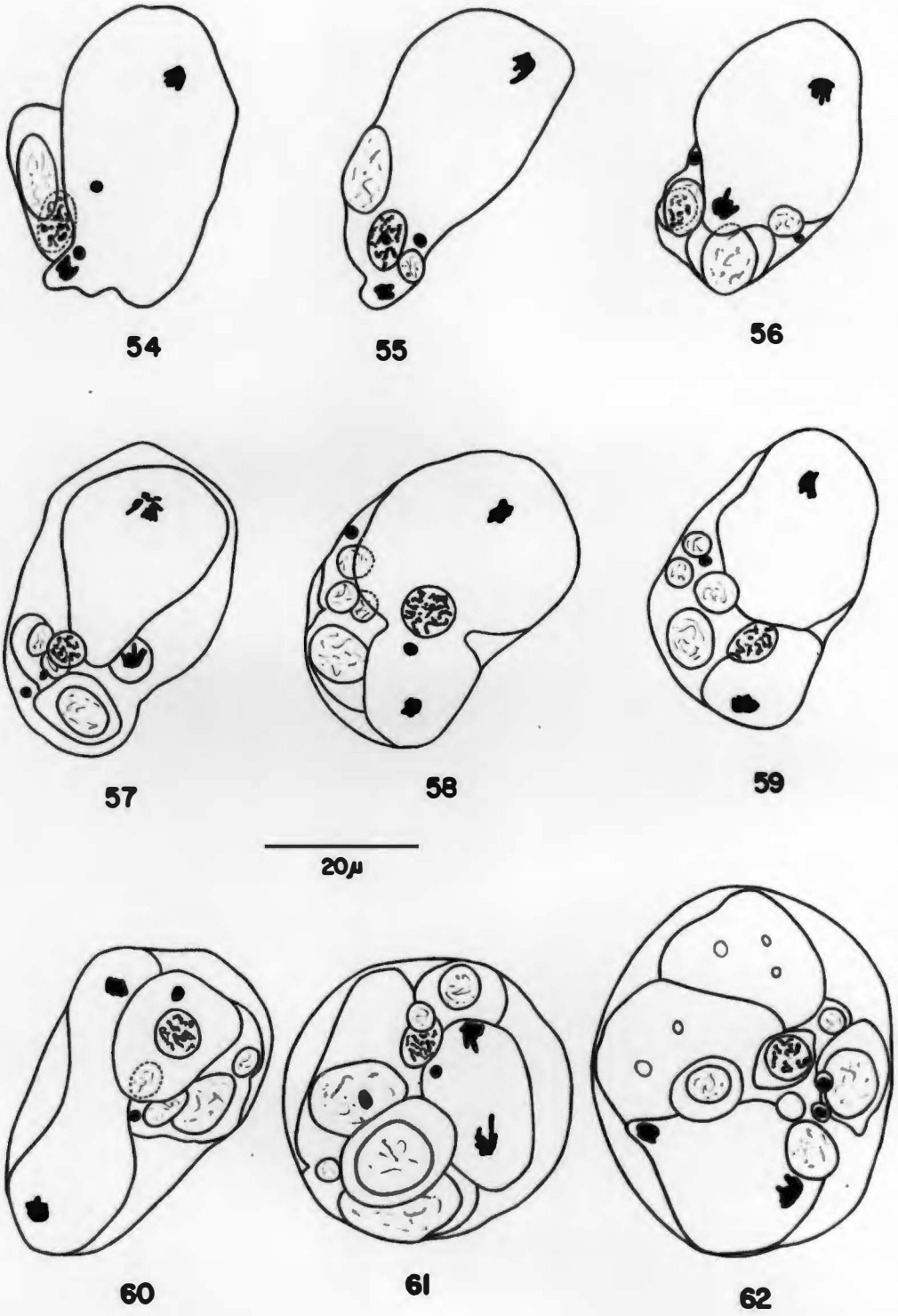

PLATE II continued

PLATE III

EXPLANATION OF FIGURES

Figures 63--125 are illustrations of embryos observed in irradiated worms. Figures are grouped according to effect instead of the quantity of radiation received or the time which elapsed after irradiation. In each illustration, the quantity of radiation and fixation time is given. Figures 82--92 demonstrate bridges of all types observed. Chromosomes of embryos in figures 93--95 resemble that condition referred to as "stickiness". Figures 99--106 present variations from the normal diploid number of twelve chromosomes. Fragmentation of various degrees is found in Figures 107--125.

Figure 63. Cleavage I. The metaphase plate has been displaced, probably by the squashing process. The diploid number of chromosomes is present. One chromosome is approximately twenty-four hours after the end-point of irradiation.

Figure 64. Cleavage III. Chromosomes of the metaphase condition. Twelve are present. One is more than twice as long as any other in the cell. 800 r at 8.35 r per minute. Worm fixed approximately twenty-four hours after the end-point of the irradiation.

Figure 65. Cleavage IV. Prometaphase. Thirteen chromosomes present. The length of the long chromosome does

not greatly exceed that of other long chromosomes of the cell. Four compact masses resembling polar bodies are present. 1200 r at 3.09 r per minute. Worm fixed approximately seven hours after the end-point of irradiation.

Figure 66. Cleavage V. Chromosomes of the metaphase condition. Sixteen chromosomal elements are present. The abnormal one is three of four times longer than any other in the cell. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 67. Cleavage stage undetermined. Probably cleavage V. Chromosomes are of the metaphase condition. For photograph and explanation see Fig. 6. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-point of the irradiation.

Figure 68. Cleavage stage undetermined. Probably cleavage V. The smaller structures within the embryo drawn in outline are nuclei. The largest is that of the smaller cell from cleavage I. The nucleus of the "attached" cell is next in order of size. The three nuclei which are approximately equal in size are those of the smaller cells of cleavages II, III, and IV. The smallest structure in outline is probably a polar body. Twelve chromosomes of the prometaphase condition are present. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-

point of irradiation.

Figure 69. Cleavage stage undetermined. Probably cleavage VI. The largest structure of the embryo drawn in outline is the nucleus of the smaller cell of cleavage V. Next in order of size is the nucleus of the smaller cell of cleavage I. The nucleus of the "attached" cell is slightly smaller and located on the extreme left of the embryo. The three smaller nuclei of about the same size are products of cleavages II, III, and IV. The two smallest structures are polar bodies. This particular embryo is unusual since two long chromosomes are present. In most examples of this abnormality, only one of these long chromosomes was observed. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-point of the irradiation.

Figure 70. Cleavage stage undetermined. Probably cleavage V. For photograph and explanation see Fig. 5. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-point of irradiation.

Figure 71. Chromosomes of Fig. 5 and Fig. 70 ranked in order of size. Compare with Fig. 16.

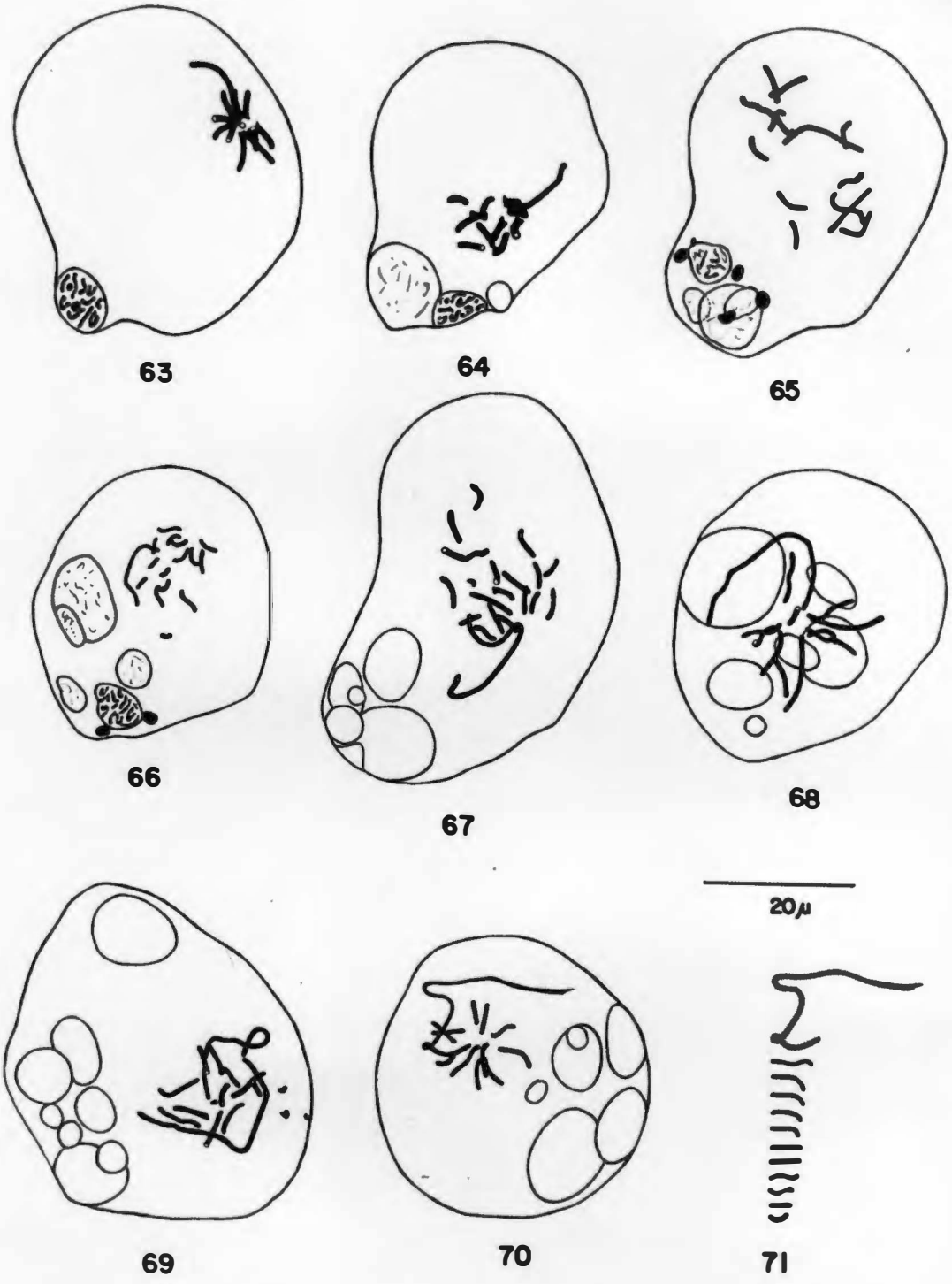

PLATE III

PLATE III continued

EXPLANATION OF FIGURES

Figure 72. Cleavage V. Spatial relations of nuclei disturbed by squashing. Twenty-two chromosomal elements of the late prometaphase type. An additional light staining mass is indicated by stipling. 1200 r at 3.09 r per minute. Worm fixed seven hours after the end-point of the irradiation.

Figure 73 Cleavage V. Chromosomes of the late prometaphase condition. One chromosome is extremely long. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 74. Cleavage IV. Metaphase chromosomes. Sixteen chromosomal elements present. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 76. Cleavage IV. Eighteen chromosomal elements of the metaphase condition are present. One chromosome is slightly longer than other long ones of the dividing cell. Note that the vitelline membrane has been broken and pulled away from the blastomeres. 5000 r at 84 r per minute. Worm fixed at eight hours after the end-point of the irradiation.

Figure 77. Cleavage V. Anaphase. Twenty-six

chromosomal elements are distributed unequally between the poles. One very long chromosome, apparently acentric, is coiled in the cytoplasm. See photograph (Fig. 7). 800 r per minute. Worm fixed approximately eight hours after irradiation.

Figure 78. Cleavage II. Anaphase. Eleven chromosomes are grouped at each pole if the long ones are included. Note that one of the polar bodies has in some way divided or is in the process of pulling apart. 1200 r at 3.09 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 79. Cleavage stage could not be determined because of the disruption of the cells by squashing. Note the distribution of the bridge to the daughter cells. The longest part remains in the larger cell while the small cell has the shortened portion. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 80. Cleavage stage undetermined. Probably cleavage VI. Anaphase. Ten chromosomes at each pole if the long ones are included. Fifteen chromosomal elements in the cytoplasm. Note that the ends of the long chromosomes located at the equatorial plane are free. No chromosome bridge is present. 800 r at 8.35 r per minute. Worm fixed eight hours after the end-point of the irradiation.

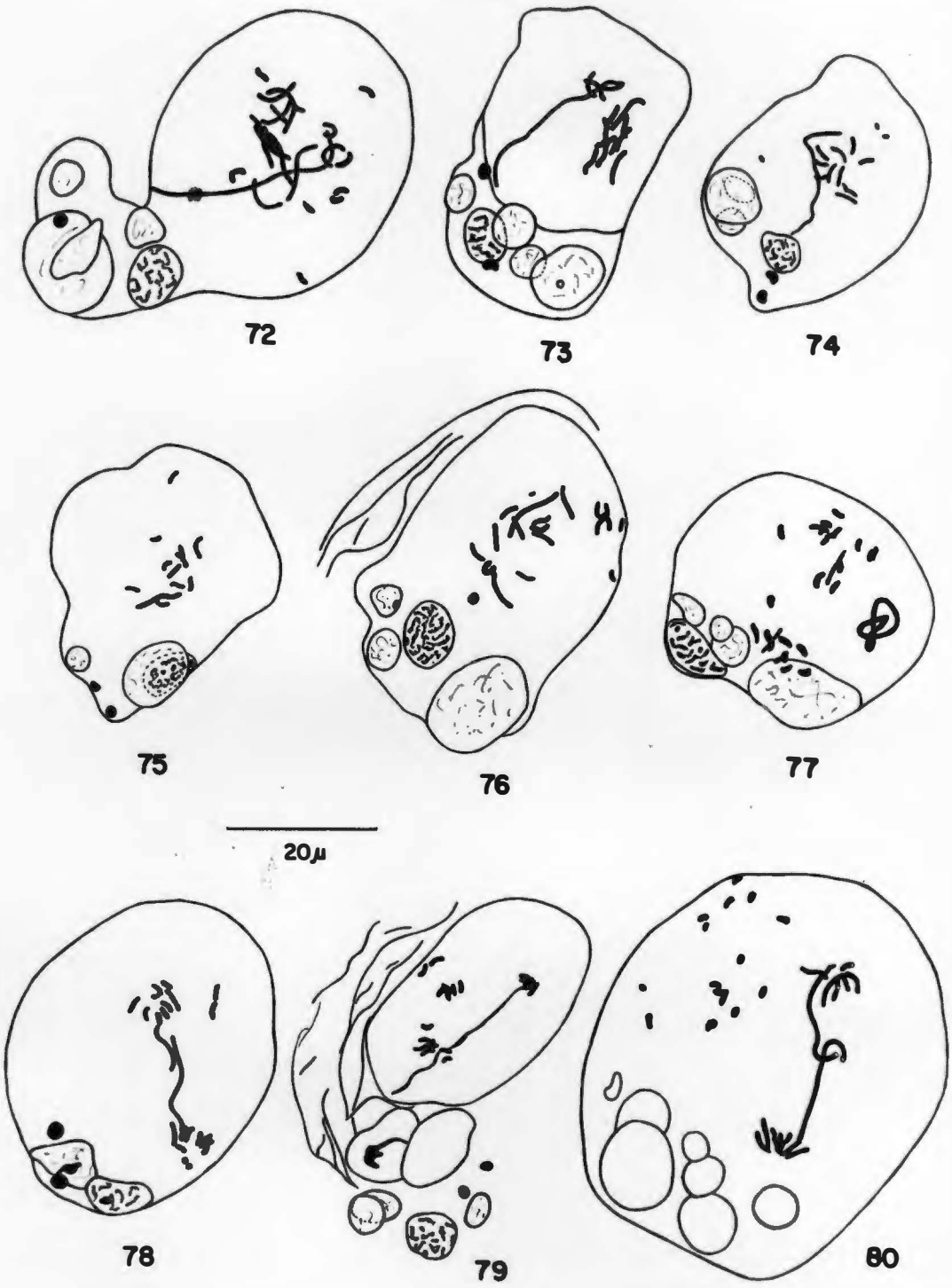

PLATE III continued

PLATE III continued

EXPLANATION OF FIGURES

Figure 81. Cleavage V. Anaphase. Twenty-four chromosomal elements and one long chromosome bridge present. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-point of the irradiation.

Figure 82. Cleavage I. Anaphase. Typical bridge observed in irradiated embryos at anaphase. 1200 r at 3.09 per minute. Worm fixed seven hours after the end-point of the irradiation.

Figure 83. Cleavage stage undetermined. Probably cleavage V. Eleven chromosomes at each pole plus the continuous bridge. Six chromosomal elements in the cytoplasm. 800 r at 8.35 r per minute. Worm fixed approximately twenty-four hours after the end-point of the irradiation.

Figure 84. Cleavage VI. Anaphase. Bridge of unequal diameter. This bridge resembles somewhat the "sticky" bridges reported for some other irradiated material. 1200 r at 3.09 r per minute. Worm fixed seven hours after the end-point of the irradiation.

Figure 85. Cleavage VI. Bridge with fragments in the cytoplasm. On the basis of the unequal diameter of the chromosomal strand, this may be a "sticky" bridge. 1200 r at 3.09 r per minute. Worm fixed seven hours after the end-

point of the irradiation.

Figure 86. Cleavage IV. Anaphase. Bridge with fragments. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 87. Cleavage IV. Late anaphase. Bridge with a few fragments in the cytoplasm. Note that one of the polar bodies appears to have divided. 1200 r at 3.09 r per minute. Worm fixed seven hours after the end-point of the irradiation.

Figure 88. Cleavage IV. Anaphase. Bridge and fragments. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 89. Cleavage stage undetermined. Probably cleavage VII. Note that the bridge is quite small in diameter. Compare with bridge illustrated in Fig. 83. 800 r at 8.45 per minute. Worm fixed approximately eight hours after the end-point of the irradiation.

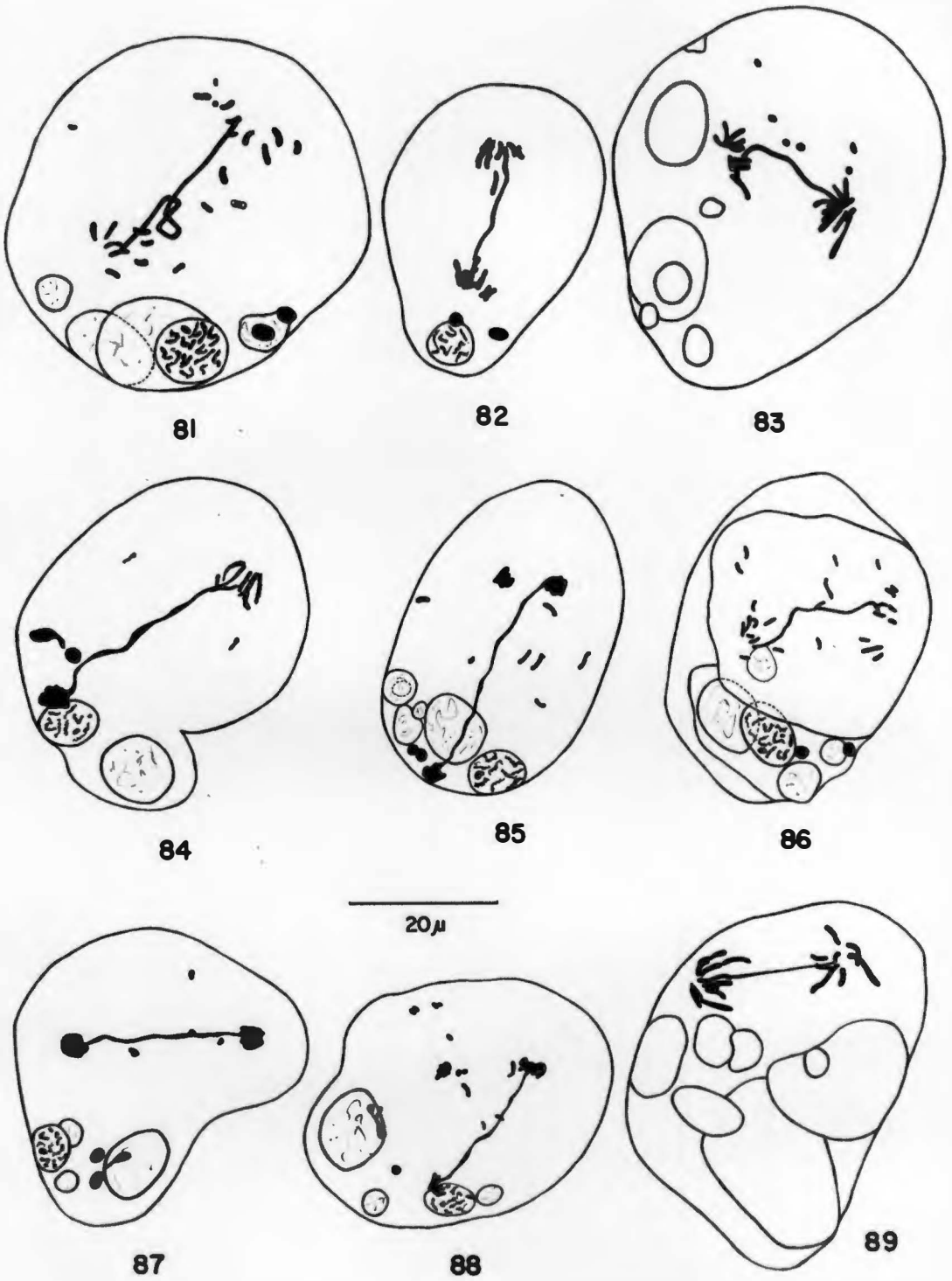

PLATE III continued

PLATE III continued

EXPLANATION OF FIGURES

Figure 90. Cleavage II. Anaphase. Broken bridge. Fragments in the cytoplasm. The bridge has broken approximately in the middle of the chromosomal strand. 1200 r at 3.09 r per minute. Worm fixed seven hours after the end-point of the irradiation.

Figure 91. Cleavage II. Anaphase. Broken bridge. This bridge has broken unequally. The large cell will receive a long part of the bridge while the small cell will receive the shortened portion. 5000 r at 84 r per minute. Worm fixed eight hours after end-point of the irradiation.

Figure 92. Cleavage V. Anaphase. Remnants of a bridge. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 93. Cleavage V. Anaphase. 1200 r at 3.09 r per minute. Worm fixed seven hours after the end-point of the irradiation.

Figure 94. Cleavage I. "Attached" cell absent. This is an extreme case of that condition resembling "stickiness" in other irradiated organisms. The bridge indicates perhaps that the stage might be anaphase. 1200 r at 3.09 r per minute. Worm fixed two hours after the end-point of the

irradiation.

Figure 95. Cleavage III. The stage may be anaphase. Much of the nuclear material appears to be clumped. 1200 r at 3.09 r per minute. Worm fixed seven hours after the end-point of the irradiation.

Figure 96. Cleavage IV. Early anaphase. Seven chromosomes are just beginning to separate. The rest of the nuclear material is clumped. 1200 r at 3.09 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 97. Meiosis. Metaphase II. Six dyads and one manad are present. This could be an irradiation effect, but it also occasionally occurs in non-irradiated material. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 98. Meiosis. Metaphase I. Note the presence of two nuclei of the "attached" cell type. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

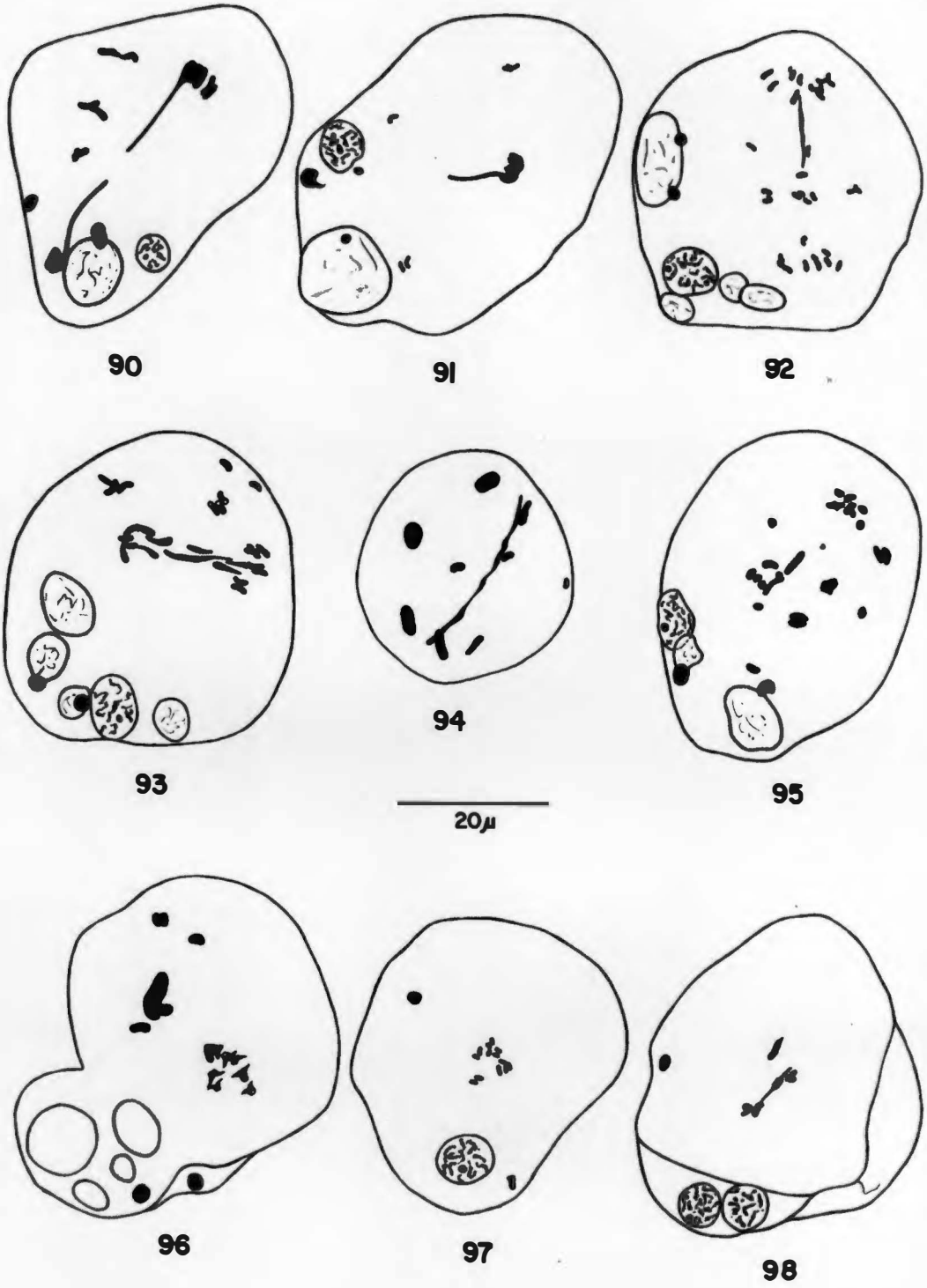

PLATE III continued

PLATE III continued

EXPLANATION OF FIGURES

Figure 99. Cleavage I. Prometaphase. Reduction in chromosome number. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 100. Cleavage II. Metaphase chromosomes. Only eleven are present. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 101. Cleavage II. Metaphase. Increase in chromosome number. The presence of an extra chromosome is occasionally observed in unirradiated material. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 102. Cleavage I. Metaphase. Thirteen chromosomes are present. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 103. Cleavage V. Metaphase. This is an extreme example of the increase in chromosome number. For photograph and explanation see Fig. 11. 800 r at 8.35 r per minute. Worm fixed approsimately eight hours after the end-point of the irradiation.

Figure 104. Cleavage stage undetermined. Probably cleavage IV. The smallest mass shown in outline is very

likely a polar body. Fourteen chromosomes are present. Four chromosome "rings" may be observed. 800 r at 8.35 r per minute. Worm fixed approximately eight hours after the end-point of the irradiation.

Figure 105. Cleavage II. Twelve chromosomes appear to be oriented in the equatorial plane. Three longer chromosomes are situated out in the cytoplasm. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 106. Cleavage IV. Note the four longer chromosomes in the cytoplasm away from the main group. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 107. Five cell stage. Chromosome fragments in the cytoplasm of a cell in interphase. The chromosomes are of the prophase degree of thickening. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

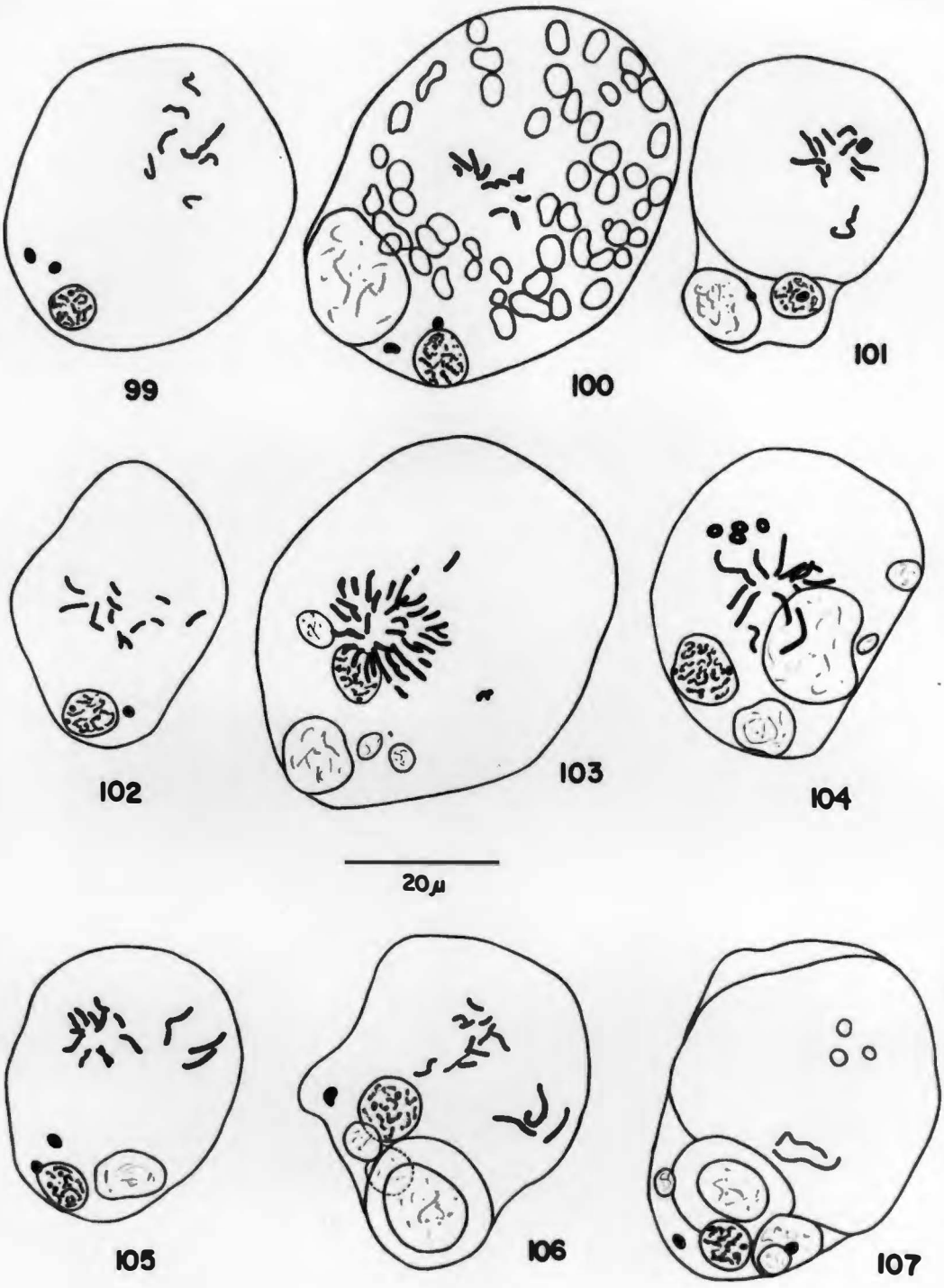

PLATE III continued

PLATE III continued

EXPLANATION OF FIGURES

Figure 108. Four cell stage. Chromosome fragments in a cell in interphase. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 109. Cleavage I. Metaphase. Twelve chromosomes and one small fragment. 5000 r at 84 r per minute. Worm fixed eight hours after irradiation.

Figure 110. Cleavage I. "Attached" cell absent. Metaphase. Fifteen chromosomes and one small fragment. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 111. Cleavage III. Early anaphase. Eighteen chromosomal elements. Those which are not separating at the ends are assumed to be fragments. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 112. Cleavage IV. Early anaphase. Twelve chromosomes are separating at one end. Others are assumed to be acentric fragments. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 113. Cleavage V. Early anaphase. Some of the chromosomal elements are pulling apart at one end. Those not separating are assumed to be fragments. 5000 r at

84 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 114. Cleavage II. Metaphase chromosomes. Note the presence of tiny fragments. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 115. Cleavage IV. An extreme case of fragmentation. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 116. Cleavage I. Example of an unexpected condition which is occasionally observed in non-irradiated embryos. Two polar bodies are present but the chromosomes are not typical of the cleavage I type. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

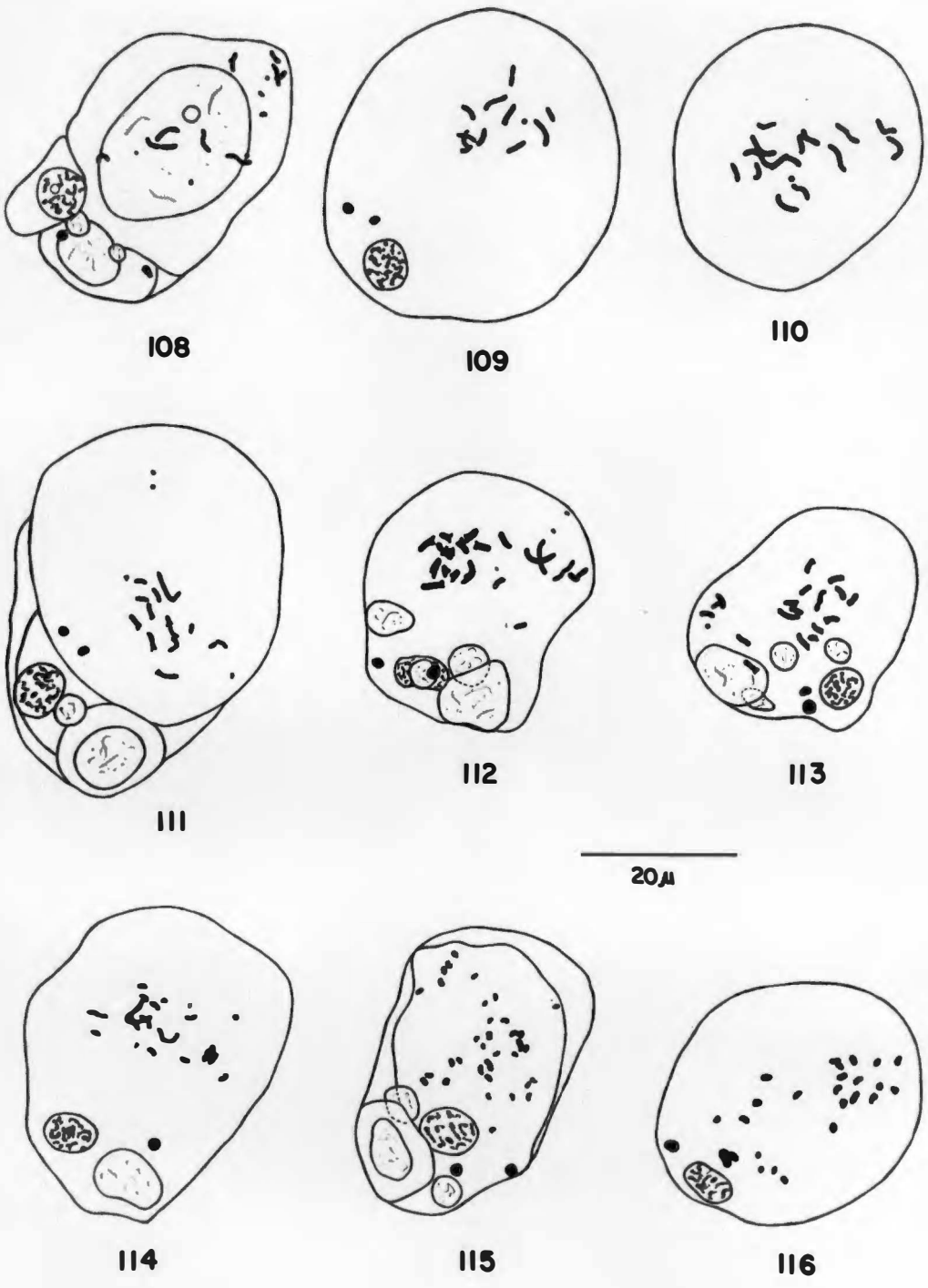

PLATE III continued

PLATE III continued

EXPLANATION OF FIGURES

Figure 117. Same as Fig. 116. In this embryo, seventeen atypical chromosomes are present. 5000 r at 84 r per minute. Worm fixed eight hours after the irradiation.

Figure 118. Cleavage IV. An extreme example of chromosome fragmentation. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 119. Cleavage IV. Early Anaphase. Fragments. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of irradiation.

Figure 120. Cleavage IV. Early anaphase. Several fragments in the cytoplasm showing no signs of separation. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 121. Cleavage III. The nucleus on the right has been displaced by the squashing. Anaphase. Four fragments in the cytoplasm. 800 r at 8.45 r per minute. Worm fixed approximately eight hours after the end-point of the irradiation.

Figure 122. Cleavage III. Anaphase. Fragments and remnants of a bridge. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

Figure 124. Cleavage III. Telophase. Example of

separation of fragments. After cytokinesis has occurred, the large cell will receive all of the fragments. 1200 r at 3.09 r per minute. Worm fixed seven hours after the end-point of the irradiation.

Figure 125. Cleavage I. Anaphase. "Attached" cell absent. Fragments in the cytoplasm. The bridge remnant at the position of the equatorial plane is not oriented as expected. This may have been produced by the squashing process. 5000 r at 84 r per minute. Worm fixed eight hours after the end-point of the irradiation.

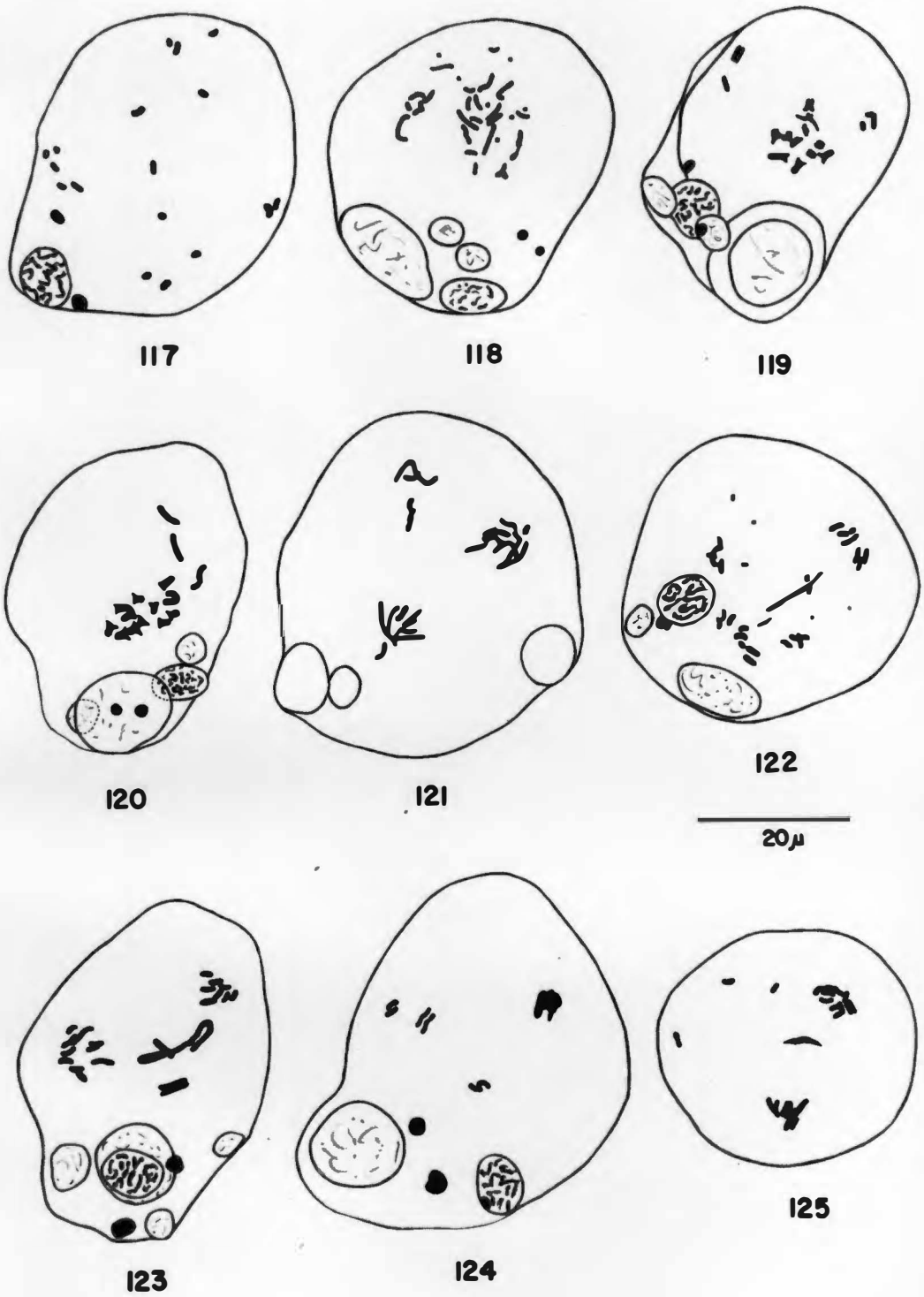

PLATE III continued